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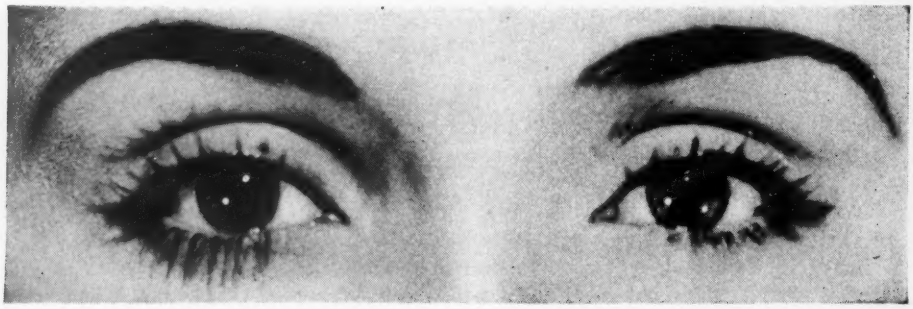
Note:

"... the nervousness and weight loss continued, and it was discovered that the patient's lack of response to sedation was because she had been surreptitiously taking 20 to 25 grains of thyroid daily throughout the summer."
(Goldfinger, D.: Ann. Int. Med. 24:701 (April) 1946.)



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VOLUME 6 OCTOBER, 1946 NUMBER 10
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THE SOURCE OF EXCESS CREATINE FOLLOWING METHYL TESTOSTERONE[1]

L. T. SAMUELS, DOROTHY M. SELLERS AND C. J. McCAULAY

From the Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah

INTRODUCTION

SINCE among the steroids only the 17-methylated compounds cause increased formation of creatine, the present studies were designed to give some information regarding the site of this action. Wilkins and Fleishmann (11) in 1941 first described the production of creatinuria in undeveloped dwarfs who were treated with methyl testosterone. Like testosterone, this compound caused increased nitrogen storage and growth, but whereas testosterone administration led to a small decrease in creatine output, methyl testosterone caused a marked increase in creatine excretion after a latent period of six to sixteen days.

Samuels, Henschel and Keys (8) in 1942 described this increased creatinuria after administration of methyl testosterone to normal young men. The output rose to as high as one gram per day in certain individuals. Not only was there a creatinuria but the blood creatine levels increased. These workers, therefore, came to the conclusion that the creatinuria was due to an increased formation of creatine rather than any change in the renal threshold.

Received for publication July 18, 1946.

[1] This work was supported by a grant from Ciba, Inc. Summit, New Jersey.

In recent work Wilkins and Fleischmann (12) found that only methylated derivatives of the androstane nucleus were effective. Methyl testosterone, methyl androstanediol-3,17 and methyl androstene-Δ_5-diol-3,17 all caused creatinuria, but ethyl testosterone was ineffective. A number of other non-methylated steroids were also found to be inactive. This work indicated that the active steroids might be associated with the methylation involved in creatine formation. It was pointed out by Wilkins and Fleischmann, however, that the amount of creatine produced would require many more methyl groups than the methyl testosterone could supply. The idea has, therefore, been advanced that methylated steroids may act as catalysts or transfer substances for methyl groups in the methylation process.

In the work of Wilkins and Fleischmann a delay has been noted in the appearance of creatine in the urine, varying from six to sixteen days after administration. The level then rises rapidly for the first 24 days and continues its upward trend for about four months after which the creatine output is maintained at a fairly constant level. Upon cessation of methyl testosterone administration, according to Wilkins and Fleischmann, there is a rise in creatine excretion for a short time followed by a rapid return to the level which existed before treatment.

Thus far, no animal other than the human being has been found which will show increased creatine excretion after administration of methyl testosterone. Dogs, rabbits, guinea pigs, rats and pigs have been tested with no effect. In our own laboratory we have failed to obtain creatinuria in either monkeys or rats. In the case of the rats, plasma and tissues have shown no evidence of any change in creatine levels. It is, therefore, necessary to use human beings in any study of creatine formation after methyl testosterone administration.

In determining the site of formation, therefore, it was necessary to select patients in which the organs most likely to be involved were sufficiently damaged to impair function seriously. Borsook and Dubnoff (3, 5) found that glycocyamine was produced by kidney tissue, and that this compound accelerated the formation of creatine by liver slices. They, therefore, advanced the hypothesis that glycocyamine is formed in the kidneys from glycine and arginine and is transported to the liver where it is methylated by methionine or other methyl donators.

The chemical cycle has been confirmed by experiments by Bloch and Schoenheimer (2), but the anatomical localization has been questioned by other workers. In fact, Borsook and Dubnoff (4) observed that creatine was formed in the kidney of mammals, but its formation was not accelerated by the addition of glycocyamine or methionine. It is possible, therefore, that the kidney as well as the liver is involved in creatine

synthesis. The precursors in the latter case either would not seem to be those postulated for the liver, or glycocyamine and methionine are supplied at an optimum rate by the kidney tissue.

We have, accordingly, studied cases of hepatic and renal disease in an effort to localize the action of methyl testosterone. Since renal function and water balance are disturbed in these conditions it was necessary to study blood levels as well as urine excretion.

METHODS

Subjects for the study were selected from the wards of the University of Minnesota hospitals and the Salt Lake County General Hospital. They were placed on a low creatine diet. Forty-eight hours later the first of three consecutive twenty-four hour urine collections was begun. These collections were made from morning to morning. At the end of each twenty-four hour collection a sample of blood was drawn. At the end of the collection period the patients were returned to the regular diet of the hospital until forty-eight hours before collection of the next sample when the low creatine diet was again resumed.

After collection of the first three urine and blood samples administration of methyl testosterone was begun. In most cases a 10 mg. tablet was taken before each meal and two before retiring, making a total of 50 mg. per day. In some cases two 10 mg. tablets were taken before each meal, thus making a dose of 60 mg. per day. A series of three consecutive urine and blood samples was collected beginning on the 9th and 19th days. In some normal cases the drug was continued for 28 days and samples were collected at the end of that time and at ten day intervals after cessation of the drug.

The plasma and urine were analyzed for preformed and total creatinine (7). The only change was to lengthen the autoclave period to forty-five minutes after it had been demonstrated that the conversion of creatine to creatinine was not complete in the time originally given. The values from each set of three consecutive samples were averaged to give the levels recorded in the graphs and tables.

RESULTS

Studies of normal subjects

The plasma and urine levels of creatine in normal women and men before, during, and after methyl testosterone administration are given in Figure 1. All of these subjects were on a low creatine diet for forty-eight hours before and during the collection of samples. In all cases no significant increase in urine creatine was observed at the end of the first ten days of treatment, but was present at the end of twenty days. On the other hand there was a small rise in plasma levels in all except two cases at

the end of ten days. The rise at the end of twenty days, however, was considerably greater.

Figure 2 illustrates the effect of oral creatine on creatinemia following methyl testosterone. If normal men consumed an ordinary meat and vegetable diet throughout the treatment period there was only a small increase in plasma levels at the end of five days but a marked one at the end of twelve days. If one gram of creatine was administered in addition to the

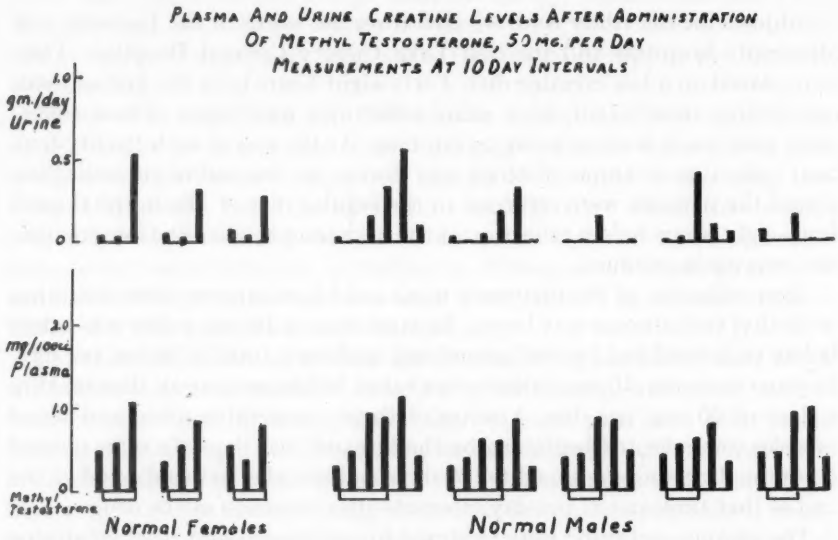

FIG. 1

normal diet the plasma level, although the same without administration of methyl testosterone as in the low creatine and ordinary diet groups, showed a marked rise within five days after administration of the steroid, and continued to rise. Apparently the greater the intake of creatine the more promptly a significant rise occurs in the plasma and in the excretion of creatine. This would indicate that methyl testosterone probably accelerates creatine formation soon after its initial administration. The excess creatine which is formed passes into the tissues where a concentration 30 to 400 times that found in the plasma exists. There is a limit, however, to the amount of creatine which the tissues can take up at this ratio. When this is exceeded, the creatine level of the blood rises rapidly until at values between 0.6 and 0.7 mg. per 100 cc. of plasma the creatine threshold is exceeded and creatinuria develops.

Studies on hepatic and renal disease

Figure 3 shows the plasma levels and urinary excretion of creatine in cases of hepatic and renal disease. In a number of other cases urine levels alone were studied but since urine levels do not reflect blood levels accurately in the presence of severe glomerular damage as seen in *Cases 5, 7* and *8*, we have not included them in the tabulation.

Hepatic disease, even when terminal, did not interfere with the rise in

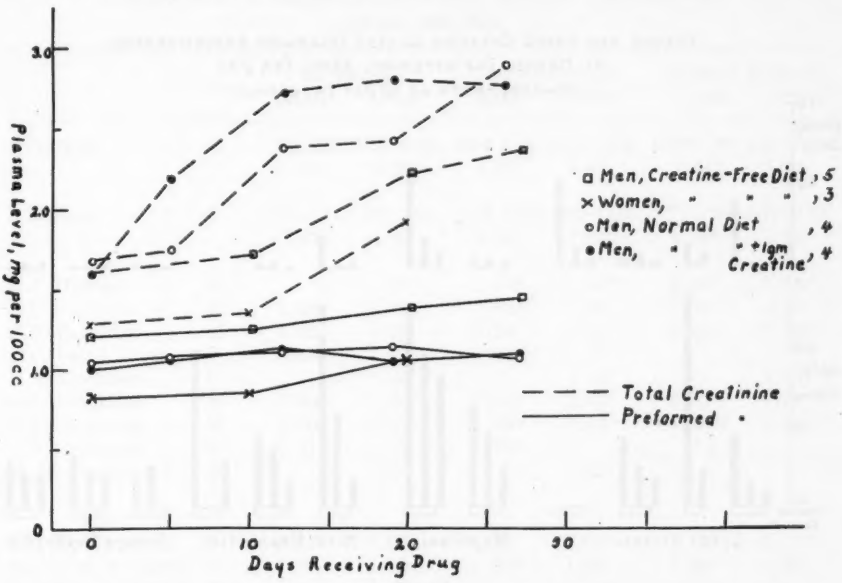

FIG. 2. Change in plasma creatine concentration during administration of 50 mg. methyl testosterone in groups with varying intakes of creatine by mouth. Averages of number of subjects indicated.

creatine output produced by methyl testosterone. Two cases of cirrhosis died while under treatment with the drug, but showed a rapid increase in creatinemia and creatinuria up to the time of death. In the one case of cirrhosis and one of fatty degeneration (these patients lived for a short period of time after tests were run), the creatine levels decreased after cessation of therapy with the drug. It seems, therefore, that if the liver is involved in the increased formation of creatine after administration of methyl testosterone, it is able to carry on this function adequately when its other functions are impaired to an extent incompatible with life.

In the cases of nephrosis studied there also was ample evidence of the

influence of methyl testosterone on creatine formation. Since in these cases the creatine levels in the blood were already somewhat elevated, the rise in plasma creatine was rapid from the beginning of treatment. Creatinuria was not so marked because of the general renal failure. It seems that the renal and hepatic damage in nephrosis, even when severe, does not prevent the action of the methylated steroids.

In the milder cases of nephritis the same situation was found. There was an immediate rise in the plasma creatine levels which was not always

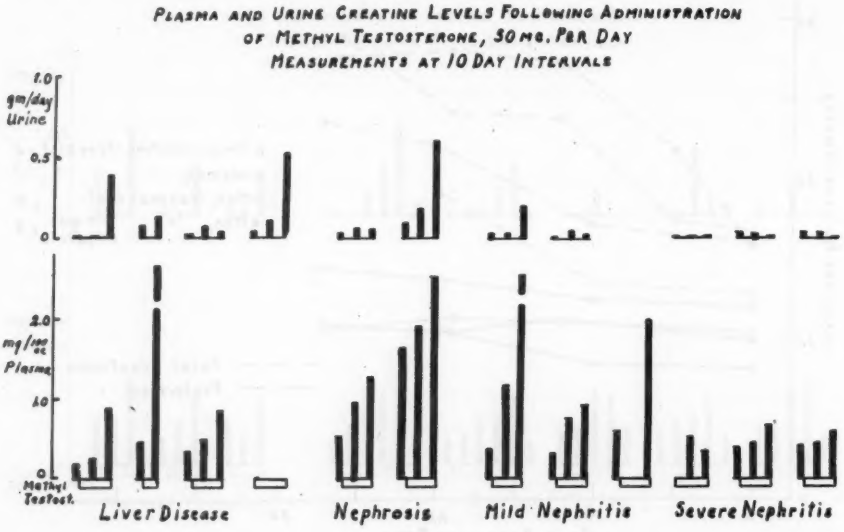

FIG. 3

reflected in urine creatine. This was not a true rise in the creatine threshold, since it probably did not represent increased absorption; instead, it represented decreased filtration.

The only condition in which there was a significant influence on creatine formation was severe nephritis. Here there was no significant rise in the plasma creatine level nor in the creatine output in the urine. There would appear to be a slight increase in the plasma creatine levels in two of the three cases illustrated in Figure 3. A study of Table 1 in which the ratio of total creatinine (creatine plus creatinine) to preformed creatinine is given will show that there was no significant rise in this ratio in these cases. The slight rise in plasma creatine was not due to an increased production of creatine but to the decrease in glomerular filtration which consequently backed up both creatinine and creatine in the blood.

DISCUSSION

The data presented here are in accord with the data of Bloch and Schoenheimer (1) that creatine from any source enters into the general creatine metabolism of the body; there was no distinction between plasma creatine due to normal endogenous metabolism, creatine produced after methyl testosterone administration, and that ingested.

TABLE 1. RATIO OF TOTAL TO PREFORMED CREATININE IN BLOOD AND URINE OF SUBJECTS TREATED FOR 20 DAYS WITH METHYL TESTOSTERONE, 50 MG. PER DAY

	Sex	Untreated		Treated 10 days		Treated 20 days	
		Blood	Urine	Blood	Urine	Blood	Urine
8 Normal	M	1.23–1.54	0.99–1.05	1.34–1.41	0.99–1.14	1.39–1.82	1.01–1.28*
		1.34	1.02	1.37	1.03	1.61*	1.08
3 Normal	F	1.42–1.71	1.02–1.06	1.46–1.67	1.01–1.03	1.73–1.99	1.22–1.33
		1.57	1.04	1.58	1.02	1.80*	1.26*
L.J. Hypopit.	M		1.20*	2.14*	1.52*	2.18*	1.68*
S.Z. Cirrhosis	M	1.29	1.02	1.45*	1.05	1.81*	1.94
P.B. Cirrhosis	M	1.32	1.03	1.33	1.00	3.24*	1.79*
J.Y. Fatty Liver	F	1.14	1.06	2.17*	1.18*		
A.R. Cirrhosis	F		1.08		1.16*		2.24*
G.R. Nephrosis	M	1.66*	1.52*	1.91*	2.06*	2.51*	3.09*
A.P. Nephrosis	M	1.30	1.05	1.52*	1.06	1.59*	1.06
J.P. Nephrosis	M	1.31	1.03	1.74*			
W.M. Nephrosis	M		1.05		1.34*		
Mild							
S.G. Chr. Nephritis	F	1.14	1.05	1.32	1.04	1.70*	1.31*
M.B. Chr. Nephritis	M	1.33	1.00	1.83*	1.04	1.81*	1.00
D.D. Chr. Nephritis	M		1.03		1.76*		
Severe							
D.H. Chr. Nephritis	M		1.01	1.23	1.01	1.28	1.03
M.R. Acute Nephritis	F	1.22	1.04	1.23	1.04	1.30	1.03
H.P. Acute Nephritis	M	1.35	1.04	1.35	0.99	1.49	1.03

* Indicates a significant increase above normal.

The course of events observed can best be explained by the assumption that creatine from any of these sources first is distributed between cells and circulating fluids at the high ratio seen under ordinary conditions. On this basis an excess production of 0.5 gm. a day could be accumulated for six days or more before the general increase in the tissues would equal ten per cent of the original concentration.

The increased concentration would then increase conversion to crea-

tinine and account for the smaller but significant increase in this component in the plasma and urine. Here again creatine from any source appears effective.

Apparently the ability to increase tissue concentrations, and perhaps also destruction, at this high ratio is soon reached, however, and as creatine production continues to increase the distribution-ratio rapidly approaches that of any solute acting under purely osmotic forces. Thus if the tissues have been saturated either because plasma levels have risen due to reduced filtration or because of ingestion the administration of methyl testosterone brings a prompt marked rise in plasma creatine levels. On the other hand, in the normal individual, the rise in plasma creatine is slow at first, but rises at an accelerated pace as treatment continues.

Our results also confirm the observations of Tierney and Peters (10) regarding the level of the renal threshold in men. In any case, except those with renal damage, whenever the plasma level exceeded 0.6 to 0.65 mg./100 cc., significant creatinuria occurred. Our observations on women, however, are not in agreement. Tierney and Peters found that orally administered creatine increased the plasma creatine levels more markedly and caused more creatinuria in women than in men. He assumed that the tissue capacity was already reached in the former sex. If this were the case in our normal female subjects, we should have expected an earlier rise in plasma and urine creatine levels than in men. This was not the case. This may be because of the limited number. None of the young women showed any significant creatinuria before administration of the drug.

It would seem that methyl testosterone action involves the kidney and requires little, if any, functioning liver tissue; otherwise one would have expected liver damange to have interfered more with creatine formation. Certainly the failure in creatine formation after renal damage supports this, although as an isolated fact this last observation would simply indicate that the kidney was involved in the formation of creatine or of its precursors. The structure of the glomerulus and the renal arginase (9) activity is markedly affected by methyl testosterone and testosterone (6), and it may well be that they also increase the enzymes involved in renal creatine synthesis; only the methylated steroid, however, will supply the methyl groups required.

There remains the possibility that creatine formation after administration of the steroid occurs in some other tissue, such as the muscle, but requires a precursor such as glycocyamine from the kidney. The storage of nitrogen after methyl testosterone would appear to be in the muscles.

SUMMARY

When methyl testosterone was administered orally to normal men or

women on a low creatine diet, the rise in plasma levels of creatine was low during the first ten days, but increased rapidly during the second ten days, exceeding the renal threshold and producing creatinuria.

If creatine were ingested or if plasma creatine levels were high due to renal disease the marked rise in plasma levels occurred sooner after beginning ingestion of the steroid. This is interpreted as indicating there is a limited capacity for storage of creatine in the tissues which can be filled by creatine from any source.

Increased creatinemia and creatinuria followed administration of methyl testosterone to patients with severe hepatic disease.

The effect of methyl testosterone on creatine formation was observed in nephrosis and mild nephritis, but was abolished in severe nephritis. It would seem that the kidney is the primary site of action or that it supplies an essential precursor to some other tissue.

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AN EVALUATION OF THE GUTERMAN PREGNANCY TEST IN CLINICAL PRACTICE[1]

H. L. REINHART, M.D. AND A. C. BARNES, M.S., M.D.

From the Departments of Clinical Pathology and of Obstetrics and Gynecology, the Ohio State University Medical School

THE evaluation of the Guterman pregnancy test here reported has two distinct features: In the first place, we have resisted the temptation to modify the technique and have used it as described by its sponsor (4, 5) Secondly, we have employed the procedure routinely and have avoided a "test-series" of cases. The selection of patients has been entirely in the hands of the house and attending staffs; when it was clinically necessary to determine whether or not a patient was pregnant by laboratory methods, this test was performed, although a Friedman test would also be carried out on request. By this means we have gained a more accurate estimate of the usefulness of the test for hospital and dispensary practice.

The technique outlined by Guterman employs the color reaction of Talbot *et al.* (9) and a modification of the extraction method of Astwood and Jones (1). Essentially it amounts to a qualitative reading of a quantitative change, and is based on the assumption that amenorrhea plus 1 mg. pregnandiol per 100 cc. urine occurs only with pregnancy and always with pregnancy. This assumption has been questioned on a quantitative basis since the isolation of pregnandiol (2, 6, 7), but the qualitative reading (*i.e., positive* or *negative*) based on the color reaction had not been suggested before, and merits consideration.

The reading of the test as recommended by Guterman was on the basis of the presence of an orange or yellow-orange color as determined by the naked eye (5). In our effort to maintain the original technique, the tests recorded here were read in this manner. With the appearance of a degree of error, however, the question immediately arises: Are the technicians reading the color change correctly? If so, the source of error lies in the test; if not, it lies in our own reading of the test.

In an effort to answer this question, we have checked the interpretations in about one-third of the cases by photoelectric colorimeter. After having been read and recorded, the sulfuric acid solution was transferred to another laboratory where it was checked colorimetrically by a different technician who was kept in ignorance of the original reading.

Received for publication August 5, 1946.

[1] This investigation was made possible by grant from the Comly Fund.

The 420 filter of the Evelyn photoelectric colorimeter was used, and a standard was prepared using 2 gm. of potassium dichromate per 100 cc. distilled water. With 10 cc. of this in a tube the galvanometer was set at 100. The sulfuric acid test solution was then substituted for the standard, and the galvanometer scale read directly. We have not attempted to translate these readings into quantitative terms, and will not so report them for three reasons: 1, the method of extraction is not complete enough to remove all urinary impurities, and the final color intensity is not dependent entirely on the amount of pregnandiol present; 2, the claim made for the procedure was that of qualitative accuracy, and it would gain nothing to criticize it on strict quantitative grounds; 3, our sole purpose in this "spot checking" was to assure ourselves that the tests were being interpreted accurately and that the source of error did not lie in our own personnel.

Our conclusions were as follows:

1. There is a wide color range in the final specimens. With this particular (optional) standard, solutions were encountered that deflected the needle off the scale at the right (less color than the standard, definitely negative) as well as to the left (strongly positive).
2. No test which had been reported *positive* had a reading in the same range as the *negative* tests.
3. The experienced technician can read the presence of the color accurately, and does not represent an appreciable source of error (an error of zero in the tests checked).

RESULTS

One hundred and thirty-one tests were performed. The follow-up in each case was made not by other pregnancy tests, but by the clinical outcome of the case. One patient could not be located for such follow-up, and the analyses reported here are based on 130 tests. Seventy-one of these patients were pregnant, and in these a *positive* test was obtained in 60 or 84.5 per cent. Fifty-nine cases were not pregnant, and 30 of these (50.8 per cent) had *negative* tests. These gross results must be corrected, however. At the onset of the series, the test was misunderstood by the house staff, and included in the early cases are nine patients who were having some vaginal bleeding. In view of the statement that it requires a *positive* test **plus amenorrhea** to make a diagnosis (4), these results must be ruled out. It must also be immediately acknowledged that a test which is not accurate in the face of vaginal bleeding is of restricted value as a diagnostic aid in hospital practice. These nine results were distributed as follows:

Pregnant with *positive* test	1
Not pregnant with *negative* test	1
Not pregnant with *positive* test	7

In reviewing the records of these patients we noted that in four cases progesterone therapy was started before the test-specimen was collected. In view of the fact that we have obtained *positive* tests in males given two 10 mg. doses of progesterone the day before the morning urine was collected, these cases must also be eliminated. All of these four patients who received progesterone had positive tests; three were pregnant and one was not pregnant.

The corrected results are shown in Table 1. It can be seen from this that the test achieved its greatest accuracy in the presence of pregnancy (83.6 per cent correct). In Table 2 the results are rearranged to provide the clinician with more information. Given either a *positive* or a *negative* report by the laboratory, there is approximately a 25 per cent chance of error in diagnosis. It should also be noted that only one of the eleven pregnant patients who had negative tests either aborted or threatened to abort, limiting the prognostic value of this procedure and casting some doubt on the diagnosis of "potential abortion" based on the results of such a determination.

TABLE 1

Pregnant	
Test positive	83.6 per cent
Test negative	16.4 per cent
Not Pregnant	
Test positive	42 per cent
Test negative	58 per cent

TABLE 2

Positive Test	
Patient pregnant	72.3 per cent
Patient not pregnant	27.7 per cent
Negative Test	
Patient pregnant	27.5 per cent
Patient not pregnant	72.5 per cent

DISCUSSION

There need be little comment on the usefulness of a test carrying this margin of error. Unfortunately, even if it is assumed that 50 per cent of this error is attributable to the performance of the test in this particular laboratory, it would still not be a procedure of sufficient accuracy for clinical adoption. While a rapid chemical determination for the diagnosis of pregnancy would be of tremendous value clinically (3), the variations in pregnandiol metabolism (7, 8) make it doubtful that an analysis of its excretion, whether qualitative or quantitative, will provide us with such a test.

CONCLUSIONS

1. The Guterman test was applied to 130 patients in hospital and outpatient practice when a laboratory test for pregnancy was indicated. A hand-picked "test-series" of cases was avoided.

2. The elimination of all patients who are having vaginal bleeding and any who may have received progesterone therapy limits the value of the test in routine hospital diagnostic use.

3. Both the *positive* and the *negative* report were subject to an error of about 25 per cent.

4. The color reaction is definite and can be read by an experienced technician. The source of error apparently lies not in the interpretation of the color reaction but in individual variations in the metabolism of progesterone, both in the pregnant and the non-pregnant woman.

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THE RELATIONSHIP BETWEEN BLOOD SUGAR AND LYMPHOCYTE LEVELS IN NORMAL INDIVIDUALS

H. FREEMAN, M.D. AND F. ELMADJIAN, M.S.

From the Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital, Worcester, Massachusetts

IN PREVIOUS investigations it has been our experience that in the majority of cases the sugar tolerance of subjects afflicted with mental disturbances is decreased as compared with that of normal subjects (4). In view of the relationship of the adrenal cortex hormone to sugar metabolism (5) it has been suspected that differences in the rate of adrenal discharge may play a part in determining these differences in the reaction to ingested sugar. Since adreno-cortical activity is reflected by the level of the absolute lymphocyte count (1) a study was made of normal subjects in whom simultaneous observations were made of the blood sugar levels and the absolute lymphocyte counts during the course of responses to ingested glucose.

A group of 21 normal subjects was studied, of whom 19 were males. These included university students and personnel of the hospital and were presumably free of any organic illness.

The routine Exton-Rose procedure (3) was followed, the glucose being given in two, 50 gram doses at half-hour intervals. The blood lymphocyte counts and blood sugar levels were measured before each dose of sugar and 30 minutes after the second. Two of the influential variables that have to be controlled in such studies are the effect of nervous tension on glucose tolerance and that of stress (6) on the lymphocyte count. Accordingly, in the first nine subjects a control day was utilized to permit an evaluation of the psychological factors incident to the test or the water administration attendant upon it. On this day, the subjects were given, at 30 minute intervals, two doses 0.06 gm. of saccharin dissolved in 275 cc. of water. Lymphocyte counts and blood sugar measurements were made before each dose of saccharin solution, 30 minutes after the second dose and again 75 minutes later. On a second day, the same procedure was repeated, except that 50 gm. of glucose in 275 cc. of water was ingested instead of saccharin. In the other 12 subjects the usual routine of the two-dose, one-hour test was followed without any control day.

The blood sugar was measured in venous blood by the Folin-Wu technic (macro-alkaline tartrate) with the addition of using a photoelectric

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colorimeter. The absolute lymphocyte count was measured from arterial blood obtained from the ear, the technic used being that described in a previous publication (2).

The results of the test on the first nine subjects are shown in Fig. 1. On the day in which saccharin is administered the blood sugars remain at a stationary level. The lymphocytes show a slight increase (200 cells) from the 30-minute to the 60-minute point and remain at that level for the next 75 minutes. This increase may represent a random variation or may be a special case of the slight upward trend which is found in normal subjects as the day progresses (2).

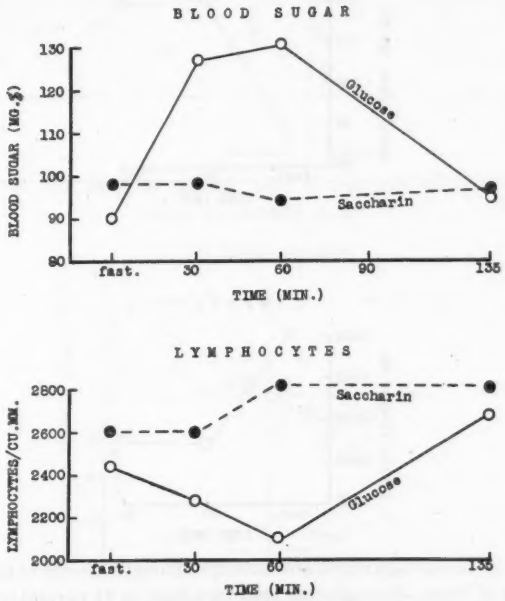

FIG. 1. Means of blood sugar and absolute lymphocyte counts obtained during the course of Exton-Rose glucose tolerance tests compared with values obtained on control days in which saccharin was administered. Nine normal subjects.

On the day in which glucose is administered, the blood sugar rises from 90 to 127 mg. per cent in the first half hour, increases to 131 mg. per cent in the second half hour and then falls to 95 mg. per cent in the next $1\frac{1}{4}$ hour. The tendency to a slight upward trend in the second half hour is probably due to the fact that the subjects may have been under some tension, which factor we have found to cause an elevation of the one hour values (4).

The lymphocyte count shows a trend converse to that of the blood sugar. The values fall at the 30 minute and 60 minute points as the blood

sugar rises and increase subsequently as the blood sugar falls. Thus the mean trends for blood sugar and blood lymphocytes are mirror images of each other. The variation in each one is beyond the range obtained during the day in which saccharin is administered. It is evident, therefore, that neither psychological factors nor water administration caused any significant variation in the blood-sugar or the lymphocyte count.

The mean values for the entire group of 21 subjects (Fig. 2) show the same inverse relationship. The sharp increase in blood sugar obtained 30

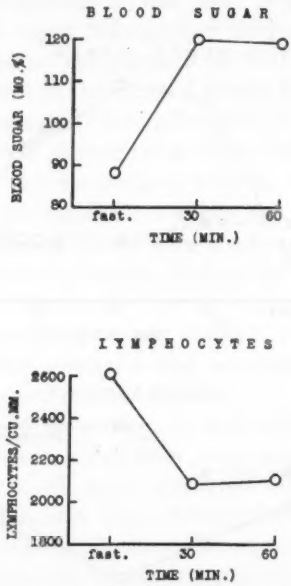

FIG. 2. Means of blood sugar and absolute lymphocyte counts obtained during the course of Exton-Rose glucose tolerance tests in 21 normal subjects.

minutes after the ingestion of 50 gm. of glucose from a fasting value of 88 mg. per cent to 120 mg. per cent is accompanied by a rapid decrease in blood lymphocytes from an initial level of 2611 per cu. mm. to 2078 per cu. mm. In the second half hour of the test, the mean blood sugar remains essentially unchanged and correspondingly so does the lymphocyte count.

In order to determine whether the individual subjects all showed the high relationship between the blood sugar values and the lymphocyte counts displayed by the mean values, a statistical study was made of the absolute changes in the blood sugar in relation to the absolute changes in the blood sugar in each individual for the first and the second half-

hour of the test. Figure 3 shows the results. In the upper figure (A) is charted the difference between the fasting and 30 minute values in blood sugar as compared with the corresponding change in blood lymphocytes. Each circle represents an individual. All the subjects showed an increase in blood sugar but in the case of one individual it was very slight. Fourteen

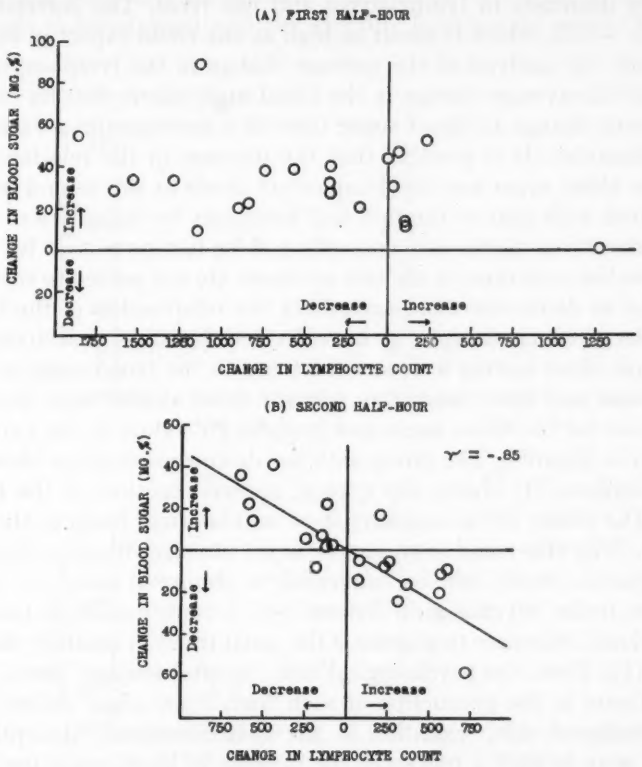

FIG. 3. Scatter diagrams illustrating the relationship between the absolute changes in blood sugar and in lymphocyte levels within each of 21 normal subjects during (A) the first half hour; and (B) the second half hour of the Exton-Rose glucose tolerance test.

of the subjects showed a decrease in lymphocytes, one showed no change and six showed an increase. The trend, of course, is in the direction indicated by the mean values in Figure 2. It is interesting, however, that there is no apparent relationship, quantitatively, between the amount of change in the blood sugar and in the lymphocyte count. Thus, although the mean values show an excellent inverse correlation between the two factors, individually the variation in one bears little relationship to the variation in the other.

On the other hand, the figure in the lower half (B) representing the changes in the second half hour shows a definite relationship between the two factors. It is evident here why the mean trends of Figure 2 are flat since the group shows upward and downward tendencies in blood sugar in about equal amounts. In this case, increases in blood sugar are accompanied by decreases in lymphocytes and *vice versa*. The correlation coefficient is -0.85, which is about as high as one could expect in biological covariation. An analysis of the average change in the lymphocytes compared with the average change in the blood sugar shows that for each 16.5 mg. per cent change in blood sugar there is a corresponding variation of 340 lymphocytes. It is possible that the increase in the relationship between the blood sugar and the lymphocyte levels in the second half-hour as compared with that of the first half-hour may be indicative of a delay in the homeostatic mechanism controlling these factors so that for at least 30 minutes the variations in the two processes are not geared to each other.

In order to determine more accurately the relationship of the lymphocytic to the glycemic changes, we have divided the subjects arbitrarily into two groups: those having a downward trend in the blood sugar at the 60 minute point and those having an upward trend at this same point. The mean values for the blood sugar and lymphocyte values in the two groups are shown in Figure 4. The group with the downward trend in blood sugar (which numbers 11) shows the typical normal reaction to the ingested glucose. The others (10 in number) show an abnormal trend in the second half-hour. Why this trend is present is as yet obscure. We have found that normal glucose trends can be converted to abnormal ones, *i.e.*, reduced tolerances, under psychological stresses (4). A confirmation of this fact is that a reduced tolerance to glucose is the usual trend in mentally disturbed subjects (4). From the psychological side, "mental tension" seems to be a possible factor in the production of such high blood sugar values (4). On the physiological side, variation in the gastrointestinal absorptive rate does not seem to play a role since the increase in blood sugar for the first 30 minutes is essentially the same in both groups. The other factors are as yet unknown.

The trends in the lymphocytes differ in the two groups of subjects. In the first group (those with a downward tendency in blood sugar) there is a marked fall in lymphocytes in the first 30 minutes and a secondary moderate rise. In the second group, there is a mild fall in lymphocytes in the first half hour, approximately 25 per cent as marked as that of the first group, and a secondary drop in the second 30 minutes. The total fall for the whole hour in lymphocytes in this group is less than that shown by the first group for the first half hour. The trends in the lymphocytes are of course mirror images of the glucose values but the difference in reaction

quantitatively is of interest since, despite the fact that the blood sugar rises are practically identical for the first 30 minutes, the lymphopenic reaction is much milder in the second group.

DISCUSSION

The data presented above show a definite inverse relationship between the levels of blood sugar and the absolute lymphocyte count. The course

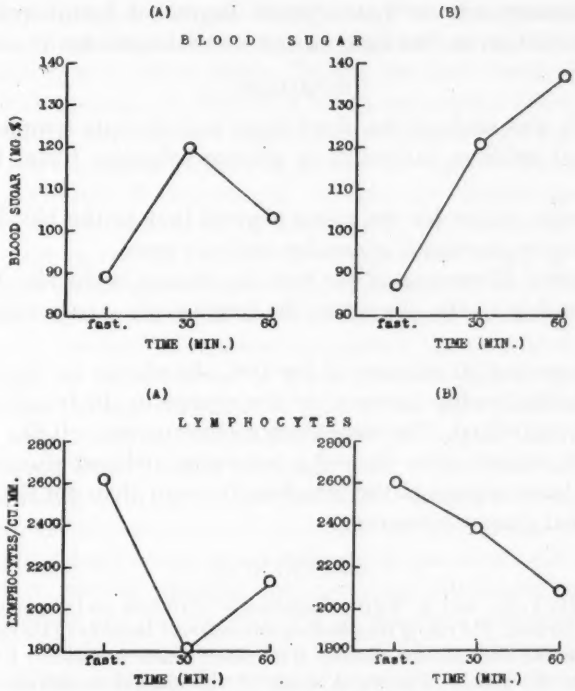

FIG. 4. Means of blood sugar and lymphocyte values obtained during the course of Exton-Rose glucose tolerance tests in 21 normal subjects divided into two groups: (A) those showing "normal" tolerance trends (11), and (B) those showing "reduced" tolerance trends (10).

of this relationship is obscure primarily because the factors controlling the shape of the glucose-tolerance trends are not definitely understood. It may be assumed that the ingestion of glucose imposes a physiological stress upon the organism. Adreno-cortical hormone is then discharged and results in a decrease in the lymphocyte count. When the blood sugar level falls, as in the second half hour of the first group (Fig. 4), adrenocortical activity

ceases and the absolute lymphocyte count rises. If the blood-sugar level continues to rise as in the second half-hour of the second group (Fig. 4), adreno-cortical activity continues and the lymphocyte count continues its fall.

The exact reasons for the lesser fall in lymphocytes in the group with the decreased tolerances are not as yet known. It is possible that the emotional tension which seems to accompany this type of tolerance curve may have already activated the adrenal cortex and to some extent caused an exhaustion phenomenon so that a lesser degree of lymphopenia results. Further speculation in the light of our small knowledge is not justified.

SUMMARY

1. A study was made of the blood sugar and absolute lymphocyte levels in 21 normal subjects subjected to glucose tolerance tests (Exton-Rose technic).

2. The mean values for the group showed that as the blood sugar rose the lymphocytes decreased in number and *vice versa*.

3. In the first 30 minutes of the test, the change in the blood sugar bore little relationship to the change in the lymphocyte count within each individual.

4. In the second 30 minutes of the test, the change in the blood sugar bore a high relationship (inverse) to the change in the lymphocyte count within each individual. The correlation coefficient was −0.85.

5. The 10 subjects who showed a somewhat reduced glucose tolerance exhibited a lesser change in the lymphocyte count than did the 11 subjects with a normal glucose tolerance.

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FURTHER STUDIES ON THE CONTROL OF MENORRHAGIA

R. B. GREENBLATT, M.D. AND H. S. KUPPERMAN, PH.D., M.D.[1]

From the Department of Endocrinology, University of Georgia School of Medicine, Augusta, Georgia

THE successful approach to the management of menorrhagia is clouded by the vast number of therapeutic measures advocated in the past. Their number is legion and runs the gamut from the speculative administration of various drugs and endocrine preparations to the *coup de grace* of the fatalist; to wit, hysterectomy. During the past decade the literature concerned with the organic therapy of functional uterine bleeding has been extensive and the promulgated methods have ranged from the administration of the time honored remedy of extract of ergot to the use of snake venom (37), vitamin B therapy (4), thyroid, the various gonadotrophic preparations, blood from lactating women (13, 14), prolactin (11, 19, 30) and, finally, any one of the various sex sterols, either singly or in combination. Inasmuch as our present knowledge of menstruation implies that it is directly under the control of the steroid hormones of the ovary, it is logical to assume that therapeutic measures to correct excessive uterine bleeding should involve the judicious use of these same steroids. So much may be accomplished with organotherapy of this nature that possible salvage of the patient may be attained without the need for surgical intervention.

The following study, which extended over a period of several years, represents an analysis of our cases of functional uterine bleeding treated by various methods utilizing steroid hormones. A comparison of the therapeutic and physiologic action of the different preparations will be outlined. In addition, a method utilizing the combined administration of testosterone propionate and progesterone will be discussed and presented as the procedure of choice. The concomitant use of these steroids has led to a rapid and efficient method of control of uterine hemorrhage on the basis of arrest of bleeding according to plan (18).

MATERIALS AND METHODS[2]

Various steroid preparations were administered to several series of patients during their phase of functional uterine bleeding. No attempt was

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[1] Roche Organon Research Fellow in Endocrinology.

[2] The testosterone propionate (*Neo-Hombreol*), progesterone (*Progestin*) and estradiol benzoate (*Dimenformon Benzoate*) used in this study were supplied by Roche Organon, Inc., Nutley, N. J. through the courtesy of Dr. B. J. Brent.

made to control the menorrhagia by any surgical procedure prior to the commencement of endocrine therapy. In the present series the following steroid hormones were employed parenterally: estradiol benzoate, progesterone, and testosterone propionate were administered singly; progesterone, in combination with either estradiol benzoate or testosterone propionate, was also employed. In addition, reference is made to our previous experiences in the therapy of functional uterine bleeding in which we implanted pellets of testosterone (15) and orally administered progesterone and anhydrohydroxyprogesterone (17, 18). Endometrial studies were made throughout this study before and after hormonal therapy when feasible in many of the patients studied.

RESULTS AND DISCUSSION

Estrogen. Many investigators are of the opinion that estrogens are contraindicated in the therapy of menometrorrhagia. Some authors believe that estrogens are not only valueless but that their use is not physiologic, since most cases of functional uterine bleeding show an hyperplastic endometrium (8, 35). On the other hand, there are many adherents to the use of estrogens in the control of uterine hemorrhage (6, 20, 28, 36). The hemostatic action of the injected steroid is thought to be an ovarian negating effect *via* inhibition of pituitary gonadotrophic hormone. It has also been suggested that the administration of exogenous estrogen controls functional uterine bleeding by raising the blood estrogen level above the so-called bleeding level (28). It has been reported that bleeding, when controlled by estrogens, will stop in an average of 4.4 days after the administration of the hormone (6).

In a previous series of cases, estrogens in the form of estradiol benzoate or dipropionate were administered in doses of from 1.66 mg. to 10.0 mg. during the phase of functional uterine bleeding. Massive doses of estrogen frequently stopped the uterine hemorrhage. However, six to ten days after cessation of therapy uterine hemorrhage recurred, and this was observed to be associated with cystic glandular hyperplasia of the endometrium. Thus, although hemostasis was adequately accomplished by the use of estrogenic substances, the subsequent withdrawal bleeding often resulted in a uterine hemorrhage as profuse as the initial episode. The prevention of estrogen withdrawal bleeding by gradual reduction in the amount of hormone administered is indeed a tedious process and still does not give the assurance that estrogen withdrawal bleeding, profuse in nature, will not ensue. It must be emphasized, however, that estrogens do have a definite place in the control of functioual uterine bleeding.

Progesterone. Progesterone has been advocated and successfully used in the control of functional uterine bleeding (2, 3, 5, 7, 9, 18, 22, 25, 26, 27, 34, 42, 43). The rationale for the administration of this steroid is based on the evidence of progesterone deficiency or persistent estrogenic endometrium in most instances of functional uterine bleeding (23). Progesterone it has been postulated, controls bleeding by the induction of a more normal state of estrogen metabolism (40). Progesterone will prevent estrogen withdrawal bleeding when administered in sufficiently high doses and will lead to a complete breakdown and sloughing of the estrogenic hyperplastic endometrium. On the basis of this it has been suggested that the withdrawal bleeding following progesterone therapy achieves the same result as a surgical curettage, and may be regarded in effect as a "medical curettage" (2). It must be emphasized that the progesterone withdrawal bleeding should not be mistaken for a prolongation or resumption of the menorrhagic syndrome but should be designated as arrest of bleeding according to plan.

Arrest of bleeding according to plan may be defined as follows: When progesterone preparations are administered for periods of from three to five successive days, either parenterally in doses of from 5 mg. to 10 mg. daily, or orally in the form of anhydrohydroxyprogesterone in doses of from 100 mg. to 150 mg. during a period of functional uterine bleeding, hemorrhage may cease, slow down or continue unabated during therapy (18). About two or three days after withdrawal of medication the bleeding suddenly may increase, continue for three or four days and then may stop abruptly or taper down and halt slowly. In all, uterine bleeding continues for six to ten days after cessation of therapy and closely resembles that of a normal menstrual period. Moreover, this latter phase will be succeeded by the desired period of amenorrhea. Unless this phenomenon, "arrest of bleeding according to plan" is appreciated, the clinician may conclude that progesterone therapy increases bleeding and accordingly is contraindicated (16).

Progesterone, on the other hand, has been placed in disrepute by a number of clinicians for the treatment of functional uterine bleeding because upon its withdrawal further bleeding ensues (22) and because its beneficial effect cannot be adequately explained (28). In addition, it has been argued that progesterone actually induces bleeding (20), since inter-menstrual bleeding may be induced on the 14th day if progesterone is administered on the 7th day through the 11th day of the menstrual cycle (44, 45). The bleeding, however, is not excessive and can be labelled as bleeding according to plan which normally ensues after progesterone therapy has been

given. Furthermore, it should be understood that progesterone does not cause bleeding when it is administered during a phase of active progestational activity, as in the post-ovulatory phase or during pregnancy (44, 45). Thus the bleeding observed after the withdrawal of progesterone therapy

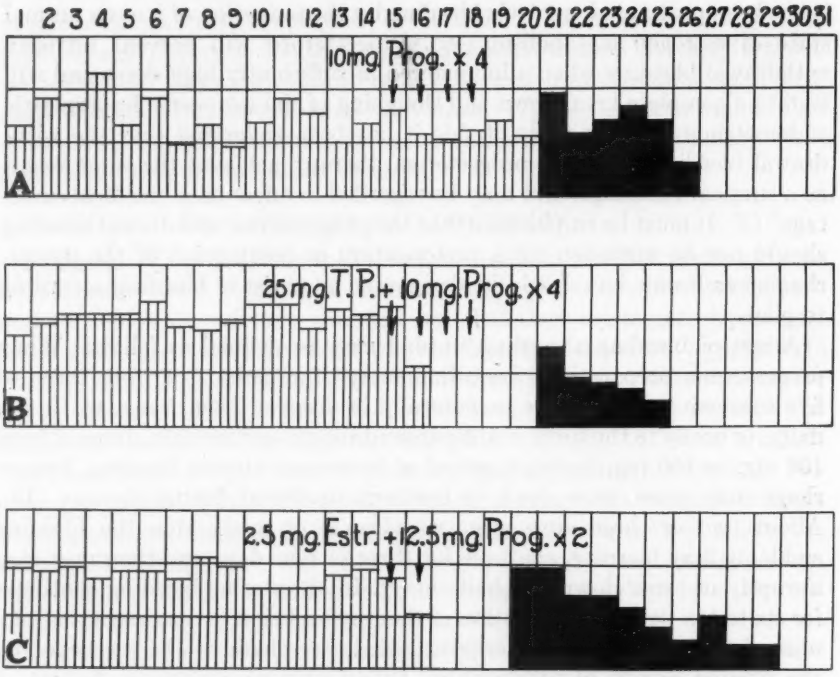

FIG. 1. Diagrammatic presentation showing effect of therapy with various steroid hormones in functional uterine bleeding.

A. Therapy = 10 mg. progesterone for four consecutive days.
B. Therapy = 25 mg. testosterone propionate administered concomitantly with 10 mg. of progesterone for four consecutive days.
C. Therapy = 2.5 mg. estradiol benzoate administered concomitantly with 12.5 mg. progesterone for two consecutive days.

Striped zone represents bout of menorrhagia.
Solid zone represents period of withdrawal bleeding.

is not analogous to that observed in menometrorrhagia but simulates the normal physiologic menstruation and will cease in a period of time approximating that of a normal menstrual period.

The general effect of administration of progesterone parenterally upon functional uterine bleeding is graphically presented in Figure 1a. As stated

above, bleeding may not be controlled during progesterone therapy. Upon discontinuation of progesterone an increase in amount of bleeding may occur but complete stoppage within six to ten days after the last progesterone injection is the usual result. Thus arrest of bleeding according to plan has occurred.

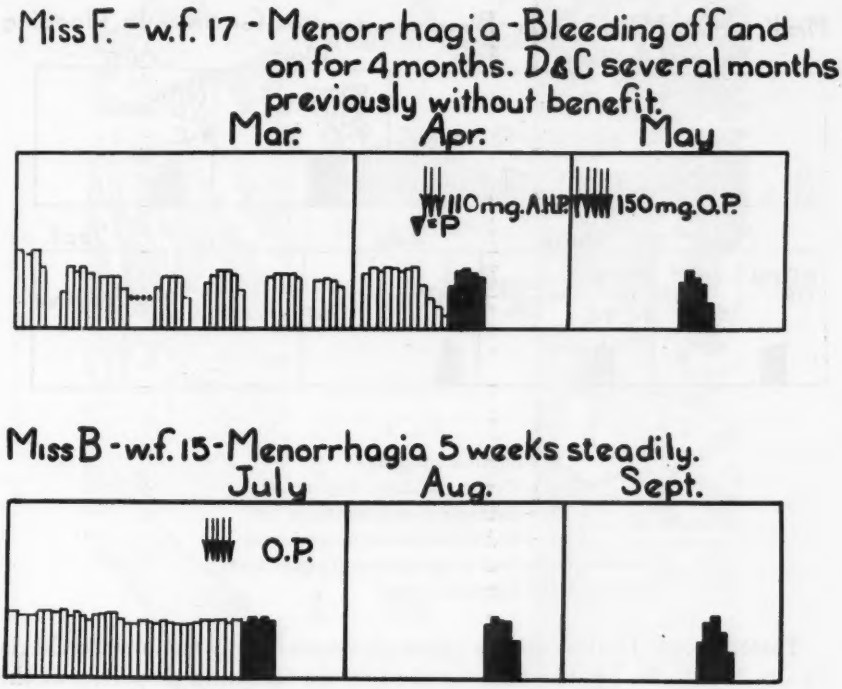

FIG. 2

∇ = Suction curettage
P = Progestinal endometrium
↓ ↓ = Days of administration of hormone
AHP = Anhydrohydroxyprogesterone
OP = Oral progesterone (linguet form)

The results observed with oral progesterone and anhydrohydroxyprogesterone have been described in previous reports (17, 18). However, three typical cases are depicted in Figures 2 and 3 where one may note that either anhydrohydroxyprogesterone or progesterone administered orally has an ameliorating effect upon the functional bleeding. Complete salvage was obtained in most instances. In several, pregnancy followed soon there-

after (Fig. 3). The average dose of progesterone employed when administered alone (parenterally) has been 10 mg. given daily for a period of from three to five days. Of 35 patients treated with parenteral or oral preparations of progesterone, 90 per cent responded in the typical pattern depicted below.

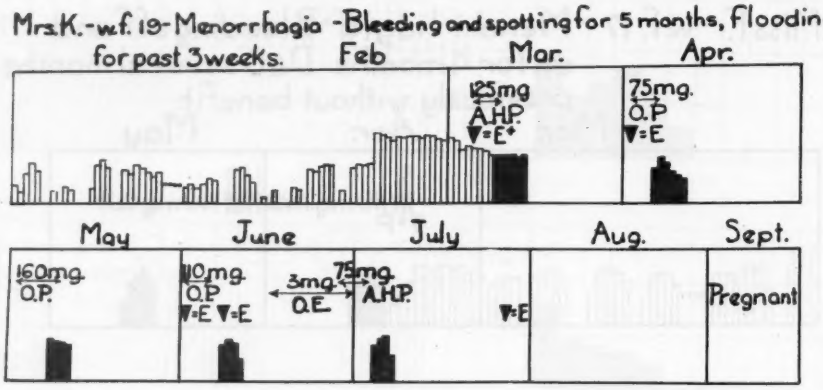

FIG. 3

▽ = Suction curettage
E + = Cystic glandular hyperplasia
E = Estrogenic endometrium
AHP = Anhydrohydroxyprogesterone
OP = Oral progesterone (linguet form)
OE = Estradiol (oral)

Testosterone. The therapeutic value of testosterone in menometrorrhagia (1, 10, 15, 33, 38, 41) has been ascribed to the following properties of the androgen: 1, estrogen neutralization (39); 2, inhibition of the pituitary gonadotrophic complex (10); 3, myometrial effects (1), which are: a, inhibition of myometrial contractions; b, stimulation of growth of myometrial elements.

The estrogen inhibiting effect of testosterone, as has been suggested (39), cannot withstand the modern concept of the physiologic action of androgenic and estrogenic substances. The nullifying effect of testosterone on the function of the female reproductive tract must be attributed to the secondary effects following the suppression of the hypophyseal gonadotrophic hormones. The mucifying action of androgens on the vaginal mucosa of the rat (39) is not due to the estrogen negating effect of testosterone but to the primary effect of the androgen on the reproductive system of the female rat (29). In addition we have noticed that estrogen

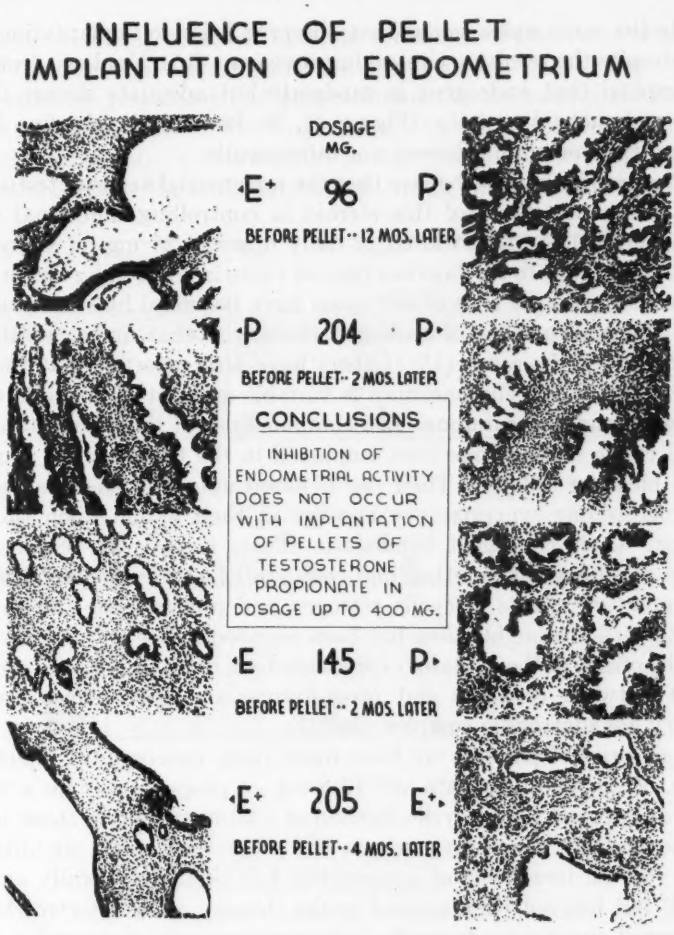
INFLUENCE OF PELLET IMPLANTATION ON ENDOMETRIUM
DOSAGE MG.
E 96 P
BEFORE PELLET-- 12 MOS. LATER
P 204 P
BEFORE PELLET-- 2 MOS. LATER
CONCLUSIONS
INHIBITION OF ENDOMETRIAL ACTIVITY DOES NOT OCCUR WITH IMPLANTATION OF PELLETS OF TESTOSTERONE PROPIONATE IN DOSAGE UP TO 400 MG.
E 145 P
BEFORE PELLET-- 2 MOS. LATER
E 205 E
BEFORE PELLET-- 4 MOS. LATER

FIG. 4. Endometriums before and after androgen therapy in a selected group of patients in whom functional uterine bleeding was successfully controlled.

applied topically to the pigmentation spot of the golden hamster in no way influences the stimulating action of simultaneously applied androgen upon the production of pigmentation and pellage changes (31, 32).

The clinical improvement following pellet implantation of testosterone in cases of functional uterine bleeding and metrorrhagia associated with uterine fibroids could not be ascribed to a pituitary inhibiting effect of the sterol. Ovarian function, as mirrored by endometrial studies, in most instances was unaltered since the endometrium was maintained in es-

sentially the same *status quo* as that observed prior to implantation of the pellet, despite the resulting clinical improvement (15). Evidence from some cases implied that androgens in moderate but adequate dosage did not inhibit endometrial activity (Figure 4). In fact, cyclic bleeding from a secretory endometrium followed not infrequently.

We, too, are inclined to believe that the myometrial actionof testosterone is the paramount effect of this steroid in controlling functional uterine bleeding (1, 15). Administration of daily doses of 25 mg. of testosterone propionate for four to six days has been of value in controlling menorrhagia. Approximately 50 per cent of our cases have benefited by such a regimen. One of the inadequacies of androgen therapy is that ultimate salvage is comparatively infrequent (15). Others have also reported that the effect of androgens on the endometrium is variable and not predictable (1, 41).

Estrogen and progesterone. The combined use of these two sterols in a form of cyclic therapy has been employed in the treatment of functional uterine bleeding (23, 24). Their use is based upon the fact that estrogen and progesterone synergize one another in their action upon the endometrium. Their combined hemostatic effect, it may be reasoned, will achieve an effect that neither one could satisfactorily accomplish alone. The regulatory effects of cyclic estrogen and progesterone therapy upon the untoward uterine bleeding has been ascribed to the estrogen employed while the return of the ovulatory cycles has been attributed to the synergism present between estrogen and progesterone and their effect upon the pituitary gonadotrophic complex (21, 23).

In our series of patients we have made daily concomitant injections of 2.5 mg. of estradiol benzoate and 12.5 mg. of progesterone[3] for a two day period as opposed to the cyclic method of administration of these steroids. The concomitant use of an estrogen and progesterone as a simplified procedure for the treatment of amenorrhea has been successfully employed (12, 46) but has not been applied to the therapy of menometrorrhagia. A summary of our composite results is diagrammatically presented in Fig. 1c. This plan differs from the cyclic mode of therapy in having a shorter period of treatment. With this procedure, cessation of bleeding usually occurs within the second day after initiation of therapy. Withdrawal bleeding follows after an interval of three or four days. The flow may continue for a period of six to ten days, with the metrostaxis in the latter half of the period being only slight in extent. Thus in contrast to progesterone alone, immediate bleeding is more quickly controlled. Amenorrhea follows the

[3] *Di-Pro Ampules,* containing 2.5 mg. *Dimenformon Benzoate* and 12.5 mg. *Progestin* were kindly furnished by Roche Organon, Inc.

withdrawal bleeding (arrest of bleeding according to plan) and persists for at least the duration of one normal menstrual period.

Testosterone propionate and progesterone. On the basis of the improvement in functional uterine bleeding achieved by the use of either androgens or progesterone alone, it was decided to incorporate the myometrial action of testosterone and the endometrial influencing effect of progesterone in a

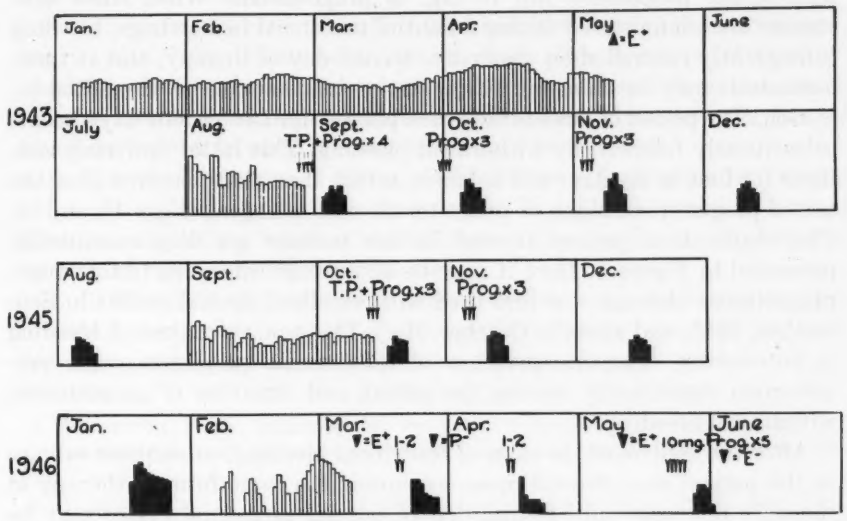

FIG. 5

Δ = D and C
▽ = Suction curettage
E + = Cystic glandular hyperplasia
E − = Hypoestrogenic endometrium
P − = Imperfect progestinal or mixed endometrium
↓ T.P. + Prog = 25. mg. testosterone propionate and 10 mg. progesterone combined therapy
↓ 1–2 = 2.5 mg. estradiol benzoate administered concomitantly with 12.5 mg. progesterone for 2 consecutive days
(Note that dilatation and curettage performed in May, 1943, controlled bleeding for only 2¼ months.)

combined form of therapy. The testosterone propionate was employed for its hemostatic propensities upon the uterus while progesterone was incorporated to induce desquamation of an hyperplastic or malfunctioning endometrium. The presumptive advantage of an androgen over that of a estrogen is that theoretically the former does not induce further hyperplasia

of the endometrium. The more obvious advantage is that combined androgen-progesterone therapy affords a "breathing spell" to the patient between the immediate arrest of bleeding and the subsequent withdrawal bleeding.

The mode of therapy that has been followed in this regimen consists of three to five consecutive daily injections of combined doses of 25 mg. of testosterone propionate and 10 mg. of progesterone. When these substances are administered during a bout of menstrual hemorrhage, bleeding is frequently controlled on the first to second day of therapy, and at times hemostasis may be observed within six to eight hours after the initial injection. The period of amenorrhea then persists for two to four days, and is subsequently followed by withdrawal bleeding. This latter flow may continue for four to six days and is less in extent than that observed after the use of progesterone alone or progesterone and estrogens (Figs. 1b and c). The results in a patient treated in this manner are diagrammatically presented in Figure 5. Here it may be noted that combined testosterone-progesterone therapy was first used with excellent clinical results in September, 1943, and again in October, 1945. The prompt control of bleeding is noteworthy. The incorporation of testosterone propionate with progesterone significantly lessens the extent and duration of progesterone withdrawal bleeding.

After the control of the siege of functional bleeding, subsequent salvage of the patient may depend upon continuing the same form of therapy at monthly intervals until resumption of normal menstrual cycles may be attained. Progesterone has proved to be most valuable and is administered for three or four days in daily doses of 10 mg. each about 21–30 days after bleeding has been controlled. If the withdrawal bleeding following these prophylactic measures is excessive, 25 mg. of testosterone propionate may be incorporated wth the progesterone to reduce the amount of flow observed after cessation of progesterone therapy. After several monthly courses of such therapy the patient is then observed for evidence of either recurrence of her functional uterine hemorrhage or the re-establishment of physiologic menstrual cycles. If there is a resumption of the former, further therapy must be instigated. In our hands we have found the combined androgen-progestin mode of therapy to yield the most promising results from the point of view of immediate hemostasis and subsequent salvage of the patient afflicted with functional uterine bleeding.[4]

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[4] In a few patients a short period of bleeding has occurred 4–7 days after cessation of the withdrawal bleeding. The bleeding was usually scanty and lasted only a few days. The physiological basis for this phenomenon is, at present, obscure.

SUMMARY

1. The examination of the endometrium soon after the arrest of menorrhagia by estrogenic therapy usually reveals an hyperplastic or a proliferative endometrium.

2. When moderate doses of androgenic therapy are employed and bleeding is arrested, the endometrium is maintained apparently in the same status as prior to therapy.

3. When progesterone therapy is used and bleeding is arrested "according to plan," *i.e.*, about seven to ten days after cessation of therapy, shedding of the endometrium frequently occurs, comparable to that which is observed in normal physiologic menstruation. However, the loss of blood during the seven to ten day period may be excessive.

4. The administration of 25 mg. of testosterone propionate and 10 mg. of progesterone daily during a four to five day period results in a rapid cessation of bleeding, which after an interval of four or five days is followed by a short bout of withdrawal bleeding.

5. In the study of functional uterine bleeding, extending over a period of many years, this method of therapy has thus far proved the most promising and ideal in the management of menorrhagia.

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PHEOCHROMOCYTOMA OF THE ADRENAL MEDULLA

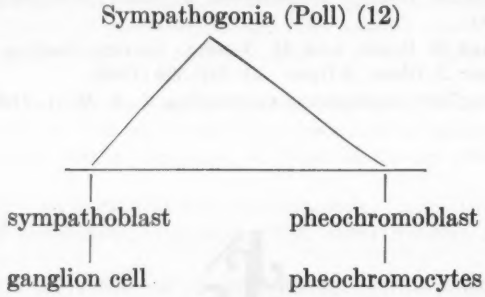
Its Role in the Pathogenesis of a Malignant Hypertension

E. L. WASHINGTON, M.D., W. P. CALLAHAN, JR., M.D., AND E. W. EDWARDS, M.D.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri

INTRODUCTION

TUMORS of the pheochromic cell arising in the adrenal medulla and producing a well established hormonal symptom-complex are occasionally encountered. The pheochromocyte or the cell of origin of this tumor is the result of differentiation of the sympathogonia or sympathetic formative cells into two systems, the future ganglion cell (nervous) and the pheochromocyte (endocrine). Sympathicoblastoma, neuroblastoma and ganglioneuroma are tumors arising in the ganglion cells in various stages of its maturation (13). They are non-hormonal and produce only signs and symptoms referable to the formation of a mass with pressure on surrounding structures. They are usually malignant and occur in infancy or early childhood (10). The pheochromocytoma arises from mature cells which have differentiated from the primitive sympathogonia to form the endocrine portion of the adrenal medulla.



The differentiation usually takes place during late fetal development and may not be complete until the time of puberty (14). The mother cells or sympathogonia are small with scanty cytoplasm and a round, deeply staining nucleus with well developed chromatin. The pheochromocytes are much larger with irregular cytoplasm which stains palely and contains many small basophilic granules. The nucleus is vesicular and ovoid with a

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prominent nucleolus. The pheochromocyte possesses the characteristic property of staining brown with chromic salts. Abnormal proliferation of the pheochromocytes or the mature cells of the endocrine series gives rise to the tumors of the adrenal medulla variously named chromaffinoma, paraganglioma or pheochromocytoma. They arise not only in the adrenal medulla but may occur wherever chromaffin tissue is present, principally the carotid body, the aortic bodies, the organ of Zuckerkandl, the sacrococcygeal region and in the retroperitoneal tissues (8). Usually they are benign tumors which remain localized, although there are on record 12 cases in which malignant transformation has been observed. When the tumor becomes malignant, the characteristic clinical symptom-complex is usually absent (9).

The tumors usually occur in adults with the peak incidence in the fifth decade. It is predominantly a disease of the white race as only 2 of 121 reported cases have occurred in Negroes.

Beer, King, and Prinzmetal (1) first demonstrated that the symptomatology was due to the presence in the blood of a pressor substance which was similar in its actions to epinephrine. Others have since supported this finding and identified the substance as epinephrine (8).

In 35 instances surgical excision of the tumor has been reported and was successfully accomplished in 27. In all, removal of the tumor resulted in complete alleviation of symptoms and there has been none in which a permanent hypertension has resulted.

The present case was considered significant because of the presence of a severe necrotizing arteriolitis in many organs of the body. Death was the result of surgical shock with no clinical manifestations of uremia. It was also unusual because of the presence of the typical clinical symptoms of the adrenal-sympathetic syndrome in association with malignant transformations in the tumor.

REPORT OF CASE

L. K. (Barnes Hospital, St. Louis, Mo., *#109286*), a 26 year old, white laborer, was admitted to Barnes Hospital on November 11, 1943 and died November 24, 1943.

Chief complaints: The patient complained of frequent attacks of severe headaches, blurring of vision, precordial distress and vomiting. He had also noticed swelling of his ankles and weight loss of 15 pounds.

Family history: His mother had high blood pressure for a number of years.

Past history: The patient was in good health until the present illness. He had been employed as a laborer for several years and was accustomed to hard work. He used no tobacco or alcohol, and no drugs other than an occasional laxative. Except for incidental injuries, he recalled no medical attention. His accustomed weight was 200 pounds. In December, 1942, he fell from a tractor and struck his head and right shoulder. He was treated in a local hospital where he remained for five or six days, but did not return to work until three weeks later. During his stay in the hospital nothing was mentioned of the presence of high blood pressure and he presumed no abnormalities were found.

Present illness: In April, 1943, he suddenly began to vomit dark green material. There was no associated nausea, but the attacks of vomiting occurred as frequently as five or six times a day. There were no other symptoms at this time. He was sent by his physician to a hospital for examination where he was told he had high blood pressure. The vomiting persisted and the patient developed severe frontal and later occipital headaches which frequently preceded the vomiting. The headaches occurred almost daily. Later the same month he awakened one morning with markedly impaired vision in the left eye which slowly improved. The right eye was unaffected. The patient had numerous nose bleeds and gingival bleeding on several occasions during his illness. Three weeks prior to his admission to Barnes Hospital he coughed up a considerable amount of dark red blood; this was unassociated with chest pain. At this time he also complained of his heart beating rapidly and vigorously. He noticed swelling of his ankles on several occasions. The patient had nocturia five or six times a night at the onset of his illness, but at the time of admission to Barnes Hospital this had ceased. He was told by his physician, however, that he had blood and albumin in his urine. During attacks the patient had noticed that his extremities would become cold and pale and then hot and flushed followed by profuse sweating. During the period of his illness he had lost 15 pounds. He became irritable and there was progressive weakness of his extremities. An accompanying letter from the patient's physician stated that during the last two months he had been given sodium thiocyanate.

Physical examination: The patient's temperature was 37° C, the pulse rate was 90 beats per minute, respirations 24 per minute, and the blood pressure was 200/150 mm. of Hg. He appeared chronically ill and the skin was pale except for some flushing of the cheeks. The eyes were normal to external examination. Opthalmoscopic examination showed recent hemorrhages at the margin of the optic discs. The gums were bleeding. The nasopharynx was normal. There was slight diminution of breath sounds over the right side of the chest anteriorly, but no other abnormalities were noted. The heart beat was forceful and thrusting with visual and tactile pulsations. The heart was enlarged to the left and there was a palpable thrill at the base. The cardiac rhythm was regular. A soft systolic murmur was heard over the mitral area and the aortic second sound was somewhat accentuated. There was some voluntary resistance to deep palpation in both kidney regions, but no definite masses were noted. The prostate was small, firm and not tender. There was no edema of the extremities. Neurologic examination was negative.

Laboratory data: The red blood cell count was 4.53 million per cubic millimeter and the hemoglobin 14.2 grams. The white blood cell count was 18,600 and the differential showed 2 basophils, 1 eosinophil, 5 stabs, 68 segmented forms, 20 lymphocytes and 4 monocytes. Examination of the urine at the time of admission showed a specific gravity of 1.011, 4+ albumin, occasional red blood cells, a few white blood cells and rare hyaline and granular casts. The Kahn reaction of the blood was negative. Examination of the stool was negative. The fasting blood sugar was 127 mg. per cent and the non-protein nitrogen of the blood was 17 mg. per cent. The total serum protein was 7.2 grams with 5.2 grams of albumin and 2.0 grams of globulin. Urine cultures showed no growth. Roentgenograms of the chest showed a focus of pulmonary infiltration at the level of the third and fourth ribs on the right. The superior margin of this region was sharply marginated and a diagnosis of "probable bronchopneumonia of the middle lobe of the right lung" was made. A barium enema was performed and reported as "indeterminate." An electrocardiogram taken shortly after admission showed "myocardial damage."

Course in the hospital: Consultation with the Department of Urology was obtained

and cystoscopic examination was performed. Examination of the urine from the right and left ureters showed 2+ albumin. There was delayed appearance of the phenosulphophthalein after intravenous injection. Intravenous pyelograms showed delayed function of both kidneys. Retrograde pyelograms showed medial rotation and downward displacement of the right kidney. The daily variations in blood pressure and pulse are shown in the accompanying chart. Repeated examinations of the urine showed persistent albuminuria and the presence of many casts. The specific gravity of the urine varied between 1.010 and 1.020. Because of the downward displacement of the right kidney and the clinical symptoms of paroxysmal hypertension a diagnosis of pheochromocytoma of the right adrenal gland was made. It was decided, in spite of the relatively unsatisfactory condition of the patient, surgical excision of the tumor should be attempted.[1] On November 24, on the thirteenth hospital day, a right lumbar incision was made from the 12th rib to the crest of the ileum. The right kidney was exposed and was found to be displaced anteriorly and downward and rotated medially. An attempt was made to explore the right adrenal gland, but when blunt dissection was attempted in this region, the patient's blood pressure fell precipitously and became unobtainable. Emergency measures consisting of stimulants, adrenal cortical extract and oxygen were administered. Exploration was discontinued and the abdomen was closed. The patient failed to respond to emergency treatment, the blood pressure was not obtainable and he expired 2½ hours after the induction of anesthesia. He was given a total of 2,000 cc. of 5 per cent glucose in saline immediately before and during the operation.

Autopsy: Washington University Department of Pathology, *# 10892*. Post mortem examination was performed one hour after death. There was a recent operative wound of the right lumbar region and a moderate amount of hemorrhage into the retroperitoneal tissues. The right kidney was displaced inferiorly and rotated medially by a large tumor which had partially replaced the right adrenal gland. The tumor was surrounded by a connective tissue capsule which also inclosed the remaining adrenal tissue. The total weight of the tumor and adrenal gland was 413 grams. Cut section of the tumor showed grayish-brown tissue which was soft, friable, and contained numerous small yellow foci of necrosis. A small angulated portion of adrenal cortex was present near the posterior margin of the tumor. No extension of the tumor through its capsule was found but it had invaded the right adrenal vein and extended into the inferior vena cava. Careful examination of the regional lymph nodes showed no gross involvement by tumor.

The weight of the kidneys was increased, the left weighing 240 grams and the right 210 grams. The surfaces were smooth and cut sections showed a normal relative thickness of the cortex and medulla. In the mucosa of the renal pelves there were numerous small hemorrhages, the largest measuring 12 millimeters in diameter.

The heart was greatly increased in size, weighing 630 gm. The pericardium was smooth and no deposits of fibrin were present on the surfaces. The myocardium of the left ventricle measured 18 mm. in diameter while that of the right ventricle measured 5 mm. The myocardium of the interventricular septum was reddish-brown and contained no foci of fibrosis. The endocardium and the valves of the heart were normal. The coronary arteries contained a few elevated yellow plaques on the intimal surface. Beneath the intima of the pulmonary artery 1.5 cm. above the pulmonic valve, there was a small focus of hemorrhage measuring 8 mm. × 2 mm.

[1] The operation was performed by Dr. Peter Heinbecker to whom we are indebted for permission to report this case.

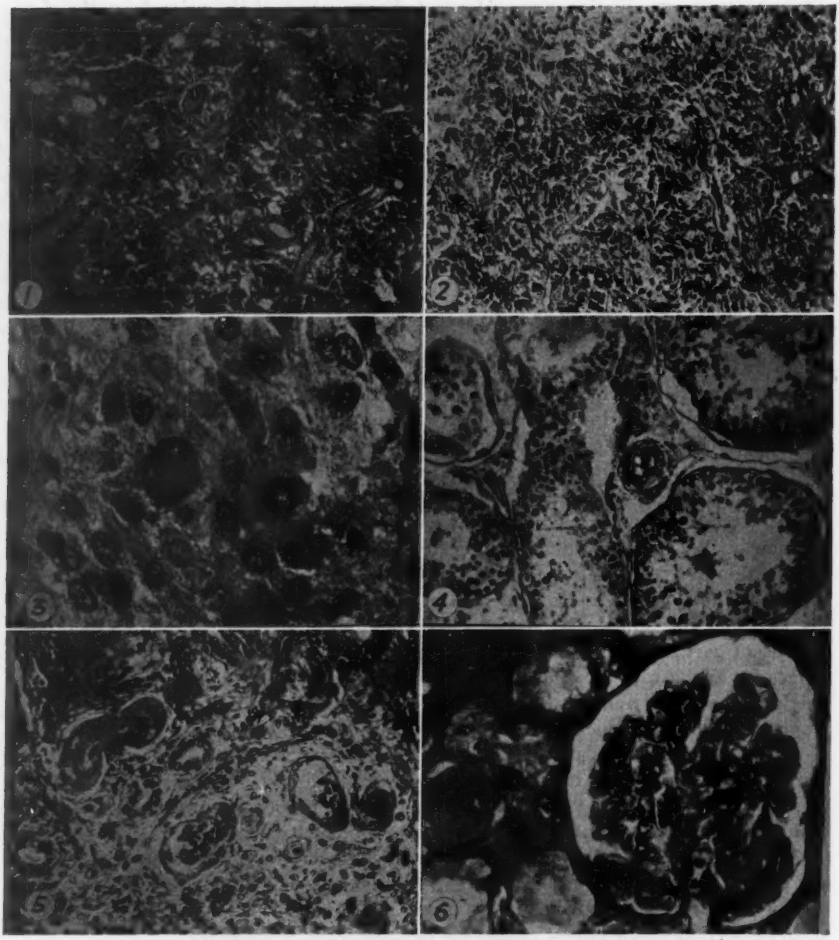

FIG. 1. Primary tumor showing pleomorphism of the cellular pattern and presence of numerous multinucleated cells. Hematoxylin eosin stain (×570).

FIG. 2. Primary tumor showing tendency toward fibrillar pattern. Multinucleated cells are also conspicuous. Hematoxylin eosin stain (×500).

FIG. 3. Eosinophilic body within the cytoplasm of a large tumor cell. The vesicular nuclei with well developed chromatin material and the granular cytoplasm are well shown. Hematoxylin eosin stain (×1100).

FIG. 4. Small arteriole within the interstitium of the testis showing hemorrhage within the wall and early necrosis. Hematoxylin eosin stain (×570).

FIG. 5. Several small renal arterioles near the corticomedullary junction. The extensive necrosis of the arteriolar wall is well shown with disappearance of a nuclei and

The lower lobes of the lungs contained a few small infarcts, the largest measuring 1 centimeter in diameter.

The other viscera contained no significant gross pathologic changes.

Microscopic examination: Blocks taken from various parts of the tumor were fixed in Zenker-formalin and in formaldehyde. Paraffin sections stained with hematoxylin-eosin and Goldner's modification of Masson's trichrome stain were examined.

The tumor consisted of large polyhedral cells which were irregular in shape and varied from 10 to 25 microns in diameter (Fig. 1). The cytoplasm was finely granular and varied in its affinity for hematoxylin, some cells staining deeply while others were only slightly basophilic. The nuclei were large and usually round although some were irregular. The chromatin network was dense and stained deeply. There was usually a prominent nucleolus. Numerous multinucleated cells were diffusely scattered throughout the tumor (Figs. 1 and 2). They contained from 3 to 8 nuclei which were hyperchromatic. The cytoplasm was abundant and stained deeply with eosin. Within the cytoplasm and occasionally within the nuclei of many of the cells were small ovoid bodies which had a homogeneous, hyaline appearance and were deeply eosinophilic (Fig. 3). Numerous mitotic figures were seen throughout the sections of tumor. In some regions the tumor cells appeared to be radially arranged around blood vessels. There were a few foci of necrosis within the tumor.

Sections of the kidneys showed increased amounts of connective tissue in both the cortex and the medulla. There was slight fibrous thickening of the peripheral layer of Bowman's capsule and of the basement membrane of the glomerular capillaries (Fig. 6). The proximal convoluted tubules were large and the epithelium was swollen. The lumina of many were filled with a granular eosinophilic substance. The cytoplasm of the tubular epithelium contained numerous eosinophilic granules and hyaline droplets. In a few of the tubules there was necrosis of the epithelium. The most striking pathologic changes in the kidneys were in the blood vessels, particularly the small arteries and arterioles. All of the arterioles observed in the sections of kidney showed marked thickening of the walls which consisted of hyalination of the media and hyperplasia of the intima. Many of the arterioles had undergone necrosis (Figs. 4 and 5). The changes in the large arteries consisted almost entirely of medial thickening and hyalination. There was very little intimal proliferation in these vessels in contrast to that seen in the arterioles. In the medulla there was slight increase in fibrous connective tissue between the tubules but the architecture was well preserved.

Sections of the pancreas showed an increase in the interlobular and interacinar connective tissue. The arterioles within the substance of the pancreas showed thickening of their walls and many had undergone necrosis. The same type of arteriolar change was present as observed in the kidney, consisting primarily of hyalination of the media and slight intimal proliferation. In some arterioles the necrosis was so extensive that the entire circumference of the wall was destroyed and the lumen was filled with necrotic material (Fig. 5).

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granularity of the cytoplasm. Goldner's modification of Masson's trichome stain (×570).

FIG. 6. Glomerulus showing minimal fibrosis of the basement membrane and of the peripheral layer of Bowman's capsule. There is no visible thickening of the efferent arteriole. In the adjacent small arteriole the nuclei have disappeared and there is necrosis of the wall with almost complete disappearance of the lumen. Goldner's modification of Masson's trichome stain (×570).

Sections of the liver showed slight dilatation of the sinusoids, particularly in the region of the central veins and congestion with red blood cells. There was no increase in the amount of periportal connective tissue. An occasional hepatic cell contained large vacuoles of fat. This fatty change was most extensive near the central veins. A few of the hepatic nuclei were vacuolated and appeared to contain glycogen.

The spleen showed evidence of passive congestion but no other pathologic changes.

Sections of skeletal muscle showed thickening of the arterioles and hyalination of the media with very little intimal proliferation. All of the arterioles in the sections of skeletal muscle showed such changes. The muscle fibers were normal.

There was slight hyperplasia of the cells of the left adrenal medulla and marked thickening of the arterioles, a few of which were necrotic. The changes in these vessels consisted primarily of medial thickening and hyalination without intimal hyperplasia. The left adrenal cortex was normal.

The lungs showed evidence of a slight passive congestion. The arterioles within the lungs did not show any of the changes observed in the other organs. There was slight atelectasis and many of the alveoli contained large macrophages which were filled with yellow-brown pigment.

Final anatomic diagnosis: Pheochromocytoma of the right adrenal (413 gm.) with partial displacement and rotation of the right kidney and with partial flattening of the upper pole of the right kidney; extension of pheochromocytoma through right suprarenal vein into the inferior vena cava; arteriolar sclerosis with necrosis of the arteriolar walls, generalized; hypertrophy and dilatation of the heart, 630 gm. (history of hypertension for 7 months); interstitial fibrosis of the pancreas; fibrosis of the islets of Langerhans, slight; recent wound of right flank (history of exploratory operation, 2½ hours); hemorrhage into the retroperitoneal tissue in the region of the right kidney; hemorrhage into the mucosa of the renal pelves; hemorrhage into the wall of the pulmonary artery; infarcts of the lungs, small; fatty metamorphosis of the liver, slight; hyaline droplets in the renal tubules.

BIO-ASSAY OF TUMOR

An extract of the tumor was prepared by the method of Folin, Cannon and Denis (5) with slight variations. Fifteen gm. of fresh tumor tissue were ground in a mortar with an abrasive and 20 cc. of 0.1 N HCl. This mixture was rinsed into an Erlenmeyer flask with 10 cc. of 0.1 N HCl and 100 cc. of distilled water and heated to boiling. To the boiling mixture was added 10 cc. of a 10 per cent sodium acetate solution and the mixture reheated to boiling. The mixture was transferred to a flask and 200 cc. of distilled water added. It was then centrifuged and the clear extract was withdrawn for physiologic tests.

A dog was prepared with ether anesthesia which was administered through a tracheal tube. A cannula was inserted into the right common carotid artery and into the femoral vein. An elastic band was placed around the thorax of the dog. Tracings of the pressure within the carotid artery and the femoral vein and of the respirations were made on a revolving kymograph. The injection of 2 cc. of the extract prepared from the tumor caused a sharp rise in blood pressure which persisted for 120 seconds be-

fore it gradually descended to the normal level. The resulting curve was nearly identical with that produced by 0.15 cc. of 1:1000 solution of epinephrine.[2] The vasopressor effect of the extract and of the solution of epinephrine was not elicited after the injection of 10 mg. of ergotoxine.

From these data it may be concluded that the tumor removed from this patient contained a substance which had a vasopressor effect on the dog analagous to that produced by epinephrine. The vasopressor effect of the extract of tumor and of the epinephrine could be prevented by the previous injection of ergotoxine.

DISCUSSION

The onset of illness in this case was typical of the paroxysms which have been observed in previously reported instances of pheochromocytoma of the adrenal gland. However, the subsequent development of permanent hypertension is unusual in patients with this disease. The demonstration of a vasopressor substance in the tumor which had an action on the blood pressure of the dog similar to that of epinephrine affords a possible explanation for the subsequent development of permanent hypertension. Goldblatt (6) has shown that elevation of the blood pressure of dogs over a period of time produces generalized thickening of the arterioles which may be responsible for the resulting permanent hypertension. The changes observed in the arterioles are comparable to those seen in man with essential hypertension. The effect of long standing vasoconstriction produced by a humoral mechanism such as epinephrine has not been thoroughly studied. Pearce and Stanton (11) have observed changes in the arteries and arterioles of rabbits subjected to repeated injections of epinephrine. The lesions were confined for the most part to the large arteries although thickening of the walls of the small arterioles was observed. The absence of any previous kidney disease or hypertension in this patient affords evidence that in the human subject the presence of large amounts of vasopressor substance in the blood over a period of time may produce arteriolar lesions which result in permanent hypertension.

The subsequent development of a malignant phase of hypertension without associated renal insufficiency is unusual. The observations of Goldblatt (7) have demonstrated that at least two factors, hypertension (mechanical factor) and uremia (humoral factor), must be present for the production of the malignant phase of hypertension and the typical pathologic changes of necrosis of the arterioles and associated hemorrhages. Dogs with a severe hypertension for as long as five years without signs of renal insufficiency or uremia did not show the clinical or pathologic changes of

[2] Parke, Davis and Company, Detroit, Michigan.

malignant hypertension. From this observation he concluded that elevation of the blood pressure alone, even to high levels for long periods of time, was not sufficient to produce the malignant phase of hypertension. Animals which did not have hypertension but which died of uremia, produced by the removal of both kidneys, likewise did not show the generalized hyalination and necrosis of the arterioles with associated hemorrhages in other organs. This showed conclusively that the accumulation of the chemical substances in the blood associated with azotemia of renal origin was not sufficient to produce the pathologic changes associated with malignant hypertension. However, dogs with hypertension which subsequently developed renal insufficiency and uremia showed necrotic arterioles and hemorrhages. That the lesions were not due to ischemia is evidenced by the absence of necrotizing changes in the arterioles of the kidney distal to the clamp. This demonstration of the pathogenesis of malignant hypertension affords the most adequate explanation of this condition in patients with hypertension of renal origin. However, it is not entirely applicable to this case because of the absence of one of the essential factors, uremia.

Wilson and Pickering (15) concluded that severe hypertension alone was sufficient to produce intimal hyperplasia and necrosis of the arterioles in the rabbit. They believed that renal insufficiency played no part in the process but based this conclusion on the absence of structural changes in the kidney and not on chemical determinations for the accumulation of the products of nitrogen metabolism in the blood. Histologic examination alone is apparently not sufficient evidence for the presence or absence of renal insufficiency.

Necrotizing arteriolar changes have not been produced in experimental animals by the injection of large amounts of epinephrine. However, in the absence of azotemia of either renal or extrarenal origin, the liberation of large amounts of pressor substance in an individual with essential hypertension may be sufficient to produce the pathologic changes of malignant hypertension. There have been no reports of the effects of injecting large amounts of epinephrine into an experimental animal with hypertension. The extremely high bursting blood pressure may under these circumstances be sufficient to produce arteriolar necrosis and associated hemorrhages without the accumulation of nitrogenous products in the blood.

Some degree of renal damage was present at the time of admission to the hospital as evidence by the presence of albumin, casts and red and white blood cells in the urine. However, the nonprotein nitrogen of the blood was 17 mgm. per cent so that there was no clinical evidence of renal insufficiency. The pathologic lesions observed microscopically in the kidneys consisted of thickening of all of the arterioles with necrosis and a

slight degree of parenchymatous degeneration of the tubular epithelium. The glomeruli showed only a slight increase in the amount of connective tissue in Bowman's capsule and in the basement membrane of the capillaries. The pathologic evidence indicated conclusively that the patient was not suffering from chronic renal disease before the onset of symptoms referrable to the adrenal tumor. Hence from these observations and the previously cited evidence that the presence of large amounts of vasopressor substances in the blood may produce changes in the arterioles which result in permanent hypertension, the continued release of this substance after the development of permanent hypertension is apparently sufficient to produce the pathologic changes associated with malignant hypertension without insufficiency.

McGavack, Benjamin, Speer and Klotz (9) studied the reported cases of malignant pheochromocytoma and found that those with widespread metastases did not present the clinical features seen in patients with the benign form of the tumor. In two cases actual hypotension was present and in none was there hypertension. They have established the criteria for denoting malignancy as the actual presence of metastases which were not present in this case. However, the extension and growth of the tumor into the inferior vena cava seems to us to be sufficient evidence upon which to make a diagnosis of malignancy. The fact that distant metastases, particularly in the lungs, had not occurred seems to be a matter of chance as all the predisposing factors were present. Certainly from the clinical standpoint the tumor was malignant as invasion and extension into the inferior vena cava made complete surgical removal all but impossible.

We have been able to find twelve cases in which the authors considered the tumors to be malignant. Braster and Keith (2) and Evans and Stewart (4) report two cases in which the typical adrenal-sympathetic syndrome was present and the tumors were considered malignant by the authors. In each instance there were local invasive tendencies without distant metastasis. These cases do not fulfill the criteria established by McGavack, Benjamin, Speer, and Klotz for malignancy. However, we believe that sufficient evidence is available in this case to classify the pheochromocytoma as malignant and that it presents the unusual combination of the adrenal-sympathetic syndrome in association with malignant transformation in the tumor.

SUMMARY

A case of pheochromocytoma of the adrenal medulla is presented. The onset of the illness was typical consisting of attacks of vomiting, headache, flushing of the face, blurring of vision and paroxysmal hypertension. The hypertension subsequently became permanent, and shortly after attempted

removal of the tumor the patient died without clinical evidence of uremia. The unusual features were the pathologic finding of a widespread necrosis of the arterioles and associated hemorrhages without evidence of uremia, and of malignant transformation in the tumor characterized by invasion of the right adrenal vein and of the inferior vena cava. Epinephrine-like substance was demonstrated in the tumor.

It is postulated that the prolonged discharge of vasopressor substance from the tumor may produce changes in the arterioles which result in permanent hypertension. The continued release of this substance after the development of a permanent elevation of the blood pressure may be responsible for the production of arteriolar necrosis and hemorrhages without the presence of uremia.

Although the tumor had undergone malignant transformation, the typical hormone symptom-complex was present.

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Letter to the Editor

To the Editor:

PROGESTERONE THERAPY OF UTERINE FIBROMYOMAS

FIBROMYOMAS have been produced in the guinea pig by estrogenic therapy. This phenomenon has been studied extensively by Lipschütz,[1] who demonstrated further that certain steroids would not only prevent formation of experimental fibromyomas but also cause regression of fibromyomas despite continued estrogenic therapy. Progesterone was by far the most effective of these steroids.

We have attempted to evaluate the effects of progesterone therapy on human uterine fibromyomas. Because it is not possible to determine small changes in size of a uterine tumor by bimanual examination alone, we have employed roentgenography with intrauterine instillation of radiopaque oil and interperitoneal injection of carbon dioxide or either of the two alone. Great care has been taken to duplicate the procedure as exactly as possible in successive studies on the same patient.

It seems apparent that if progesterone is to have a beneficial effect on human uterine fibromyomas, it will most likely be in patients who are still menstruating regularly. The three patients who are the subjects of this communication were selected because: 1, they had uterine fibromyomas which could be well outlined by one or both of our roentgenographic procedures; 2, they were menstruating regularly; 3, they were cooperative enough to permit thorough and repeated study; and 4, each understood that it was imperative to have a hysterectomy immediately after the conclusion of the therapeutic and diagnostic studies.

In addition to the effect of progesterone on the size of the fibromyomas, we observed its effects on the urinary excretion of gonadotrophic hormones and 17-ketosteroids and the histologic picture of the endometrium and mammary gland. However, this communication is concerned only with the effects on the size of the fibromyomas.

Three patients were given daily intramuscular injections of 20 mg. of progesterone in 2 cc. of peanut oil. The progesterone was administered for 39, 61 and 47 days respectively.

The therapy produced no change in the size of the fibromyomas. The

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[1] Lipschütz, A. Induction and Prevention of Abdominal Fibroids by Steroid Hormones, and Their Bearing on Growth and Development. *Cold Spring Harbor Symposia on Quantitative Biology 10:* 79–89 (1942).

size and contour at removal were as expected from the roentgenograms made with contrast medium.

The purpose of this note is to emphasize the necessity of adequate, controlled observation by objective means before conclusions may be drawn concerning this exceedingly important subject. To illustrate and emphasize this point, we felt that by bimanual examination there was a definite reduction in the size of the fibromyoma in one patient but roentgenograms with contrast medium showed no such change. Furthermore, it is our feeling that until it has been definitely demonstrated that progesterone does have a salutary effect on human uterine fibromyomas, it is essential to complete all studies of the effect of hormonal therapy on uterine tumors by histologic examination.

Our studies are in progress; this note was prompted by the recent report of Goodman[2] in this journal in which he described shrinkage of uterine tumors due to progesterone. His data were entirely subjective. Since objective technics are available, we would emphasize their importance in these studies.

ALBERT SEGALOFF, M.D.
J. C. WEED, M.D.
WILLIAM PARSON, M.D.
July 13, 1946.

From The Alton Ochsner Medical Foundation, The Ochsner Clinic and the Departments of Medicine and Gynecology, Tulane University of Louisiana School of Medicine, New Orleans.

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[2] GOODMAN, A. L. Progesterone Therapy in Uterine Fibromyoma. *J. Clin. Endocrinology 6:* 402–408 (May, 1946).

Association Announcement

Nominations for Awards of the Association

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. These include the Squibb and Ciba Awards which have been given in the past and a new award which will be known as the Ayerst, McKenna & Harrison Fellowship in Endocrinology, to be given for the first time in 1947. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary. A nomination should be accompanied by a statement of the importance of the nominee's contributions to endocrinology and by a bibliography of his most important papers with reprints if possible. Application forms may be obtained from the secretary and all nominations should be sent to Dr. Henry H. Turner, Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma, not later than March 15, 1947.

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of $1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner, in 1941 to Dr. Philip E. Smith, in 1942 to Dr. Fred C. Koch, in 1944 to Dr. Edward A. Doisy, in 1945 to Dr. E. C. Kendall, and in 1946 to Dr. Carl G. Hartman. No award was made in 1943. No age or other special limitation is stipulated by the Donor of the Award. So far it has been given for long-continued work of a high quality.

THE CIBA AWARD

The Ciba Award was established in 1942 and is to be given to an investigator not over 35 years of age. No recipient was selected in 1943. In 1944 the Award was given to Dr. E. B. Astwood, in 1945 to Dr. Jane Anne Russell and in 1946 to Dr. Martin M. Hoffman. The Award is for $1,200.00. If the recipient chooses to use the Award to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to $1,800.00.

THE AYERST, McKENNA & HARRISON FELLOWSHIP

This award was authorized by the Council at the 1946 meeting in San Francisco and will be presented for the first time at the 1947 annual meeting in Atlantic City. The annual stipend is $2,500.00 and may be renewed at the discretion of the Committee on Awards. Applicants for this fellowship shall fulfill the following requirements:

1. They must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed requirements for their Ph.D. or M.C.D. degree.
2. They must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals.
3. They must submit a program of proposed study.
4. They must indicate one or more institutions where the proposed program shall be carried out.
5. They must submit statements of approval from the investigators with whom they propose to conduct their research.
6. They must serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Association Notice

The 29th annual meeting of the Association for the Study of Internal Secretions will be held Friday and Saturday, June 6th and 7th, 1947, in the Viking Room of Haddon Hall Hotel, Atlantic City, New Jersey, preceding the Centennial meeting of the American Medical Association.

Members are urged to make reservations immediately inasmuch as the hotels expect to be filled to capacity. Make your reservations directly with Chalfonte-Haddon Hall advising them of the accommodations you wish. Rates are as follows:

	Chalfonte	Haddon Hall
Single room with bath:	$6, $7, $9	$7, $8, $10
Double room with bath (without ocean view):	$8 and $10	$10 and $12
Double room with bath (side ocean view):	$12	$14
Double room with bath (ocean front):	$14 and $16	$16 and $18

Make your reservations now and avoid disappointment—remember, you can always cancel them at a later date.

Those wishing to present papers should send the title of the paper and four copies of a comprehensive abstract to the president, Dr. Fuller Albright, Massachusetts General Hospital, Boston, Massachusetts, at their earliest convenience. Abstracts submitted should be in proper form for printing in the program. Not more than the first two hundred words can be included in the printed abstract.

Further information regarding the meeting will be forthcoming at an early date.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor; D. A. McGINTY. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

ENDOCRINE GENERAL

LI, C. H., C. KALMAN, H. M. EVANS AND MIRIAM E. SIMPSON. The effect of hypophysectomy and adrenocorticotropic hormone on the alkaline phosphatase of rat plasma. *J. Biol. Chem. 163*(3): 715–721. (1946).

The bony changes which occur in hypophysectomized rats suggested a study of phosphatase since this enzyme is intimately related to changes in bone. Plasma alkaline phosphatase decreased progressively after hypophysectomy in male and female rats 40 days of age. In normal and hypophysectomized rats, administration of adrenocorticotropic hormone in doses causing adrenal hypertrophy produced a decrease in the plasma alkaline phosphatase. Since desoxycorticosterone acetate produces an increase and corticosterone a decrease in the phosphatase of rat femurs, it is concluded that adrenals caused to hypertrophy by adrenocorticotropic hormone secrete mainly, if not wholly, steroids with an oxygen atom on carbon atom no. 11 rather than substances akin to desoxycorticosterone.—*H.L.M.*

NELSON, MARJORIE M. AND H. M. EVANS. Pantothenic acid deficiency and reproduction in the rat. *J. Nutrition 31* (4): 497–507 (1946).

The authors studied the effect of purified diets deficient in pantothenic acid on reproduction in normal adult female rats. When the experimental diets were instituted on the 13th day of gestation, no difference was noted between the control and experimental groups as far as the average number of young born and their average weight at birth. When the experimental diets were started on the first day of gestation, one-third of the implantations were resorbed. Also the average weight of the young at birth and the average number of young per litter were decreased. When the experimental diets were started 16–23 days before mating, one-third of the animals failed to have implantations, and 50–100 per cent of the implantations were resorbed. From those animals with implants that were not resorbed, the number of young per litter as well as their average birth weight was decreased. The growth and survival of the young borne by the pantothenic acid deficient mothers were decreased in proportion to the duration of the deficiency. Diets restricted in calories (to 69 per cent of normal intake) with adequate amounts of pantothenic acid did not interfere with reproduction.—*H.S.G.*

SYDENHAM, A. Amenorrhoea at Stanley Camp, Hong Kong, during internment. *Brit. Med. J. 2:* 159 (1946).

A survey of 655 British women interned in Stanley Camp, seven months after the fall of Hong Kong, showed that 60.5 per cent of the women between the ages of 15 and 45 years were suffering from amenorrhea, 53.67 per cent having had amenorrhea for more than three months. Of the 234 women with amenorrhea all except six had begun menstruating by May 1943. Because of the early development of this wave of amenorrhea, it was felt that emotional shock must have been the primary cause. Other symptoms of dietary deficiency were not obvious at that time.

A second wave of amenorrhea developed in the early months of 1944 when the protein intake was severely lowered by the removal of meat from the diet. Undernutrition was apparently the primary factor at this time. Many of the young women who developed amenorrhea showed a gain in weight of flabby fat while normal individuals were losing weight on the same diet. It was thought, therefore, that thyroid deficiency was also produced.—*L.T.S.*

PANCREAS

BARBOSA, J. J. DEC., M. B. DOCKERTY AND J. M. WAUGH. Pancreatic heterotopia. Review of the literature and report of 41 authenticated surgical cases, of which 25 were clinically significant. *Surg., Gyn. & Obst. 82:* 527–42 (1946).

Hypoglycemia and hyperinsulinism have been observed in association with heterotopic pancreatic tissue presenting both benign and malignant change in its insular portion. If exploration is being carried out in a case of hypoglycemia with a definite "Whipple's triad" and, after a thorough search, no tumor is found in the pancreas, the surgeon should look for heterotopic pancreatic tissue. The most common locations are the stomach, duodenum and jejunum in that order.—*J.M.*

HAIST, R. E. Carbohydrate metabolism in traumatic shock. *Am. J. Digest. Dis. 13* (5): 152–155 (1946).

The author observed animals whose hind limbs had been traumatized by pressure cuffs (dogs) and metal clamps (rats), shock being studied when these traumatizing agents were removed. The shocked animals showed high blood sugars and low levels of liver and muscle glycogen. The muscles of the injured extremities contained no detectable glycogen. Glucose administration led to high blood sugars over a prolonged period without affecting the glycogen levels. Insulin lowered the blood sugar but did not increase muscle or liver glycogen. Fructose raised the levels of blood glucose but did not affect the liver glycogen values. The turnover of high energy phosphorus compounds (adenosine triphosphate and phosphocreatine) was normal in the unclamped extremities. The author concluded that, since reclamping reversed most of the changes noted, some change had occurred in the damaged tissue which affected the activity of parts remote from the site of injury.—*H.S.G.*

MIRSKY, I. A. What is the cause of diabetes mellitus in man? *Am. J. Digest. Dis., 13* (5): 130–136 (1946).

The roles of pancreatic insufficiency and insulin insufficiency in causing diabetes

mellitus in the human were discussed. The author pointed out that since the pancreas in 25 per cent of diabetics showed no morphologic evidence of damage, pancreatectomy in man led to an insulin requirement much less than that observed in many human diabetics, and since the islets of Langerhans are hyperplastic in cases of acromegaly with diabetes mellitus, the pancreatic insufficiency hypothesis is invalid. However, because there are tissue proteinases or insulin antagonists which may increase the utilization, destruction or inhibition of insulin and thus produce decreased amounts of circulating insulin, the author felt insulin insufficiency to be a more likely cause for diabetes mellitus in humans. The conclusion reached was that the etiology of diabetes mellitus in man is unknown.—*H.S.G.*

MOSENTHAL, H. O. AND EILEEN BARRY. Evaluation of blood sugar tests: Significance of the non-glucose reducing substances and the arterio-venous blood sugar difference. *Am. J. Digest. Dis. 13* (5): 160–167 (1946).

The difference between the values obtained by the Folin-Wu method of blood sugar determination and the 'the true blood sugar' method of Lauber and Mattice was used as a measure of non-glucose reducing substances in the blood. In 200 cases (normal and diabetic) the non-glucose reducing substances varied from one to 78 mg. per 100 cc. (38 per cent of the cases were above the usually accepted limit of 30 mg. per 100 cc.). When glucose tolerance tests were performed, the non-glucose reducing substances fluctuated markedly and unpredictably. Therefore, the authors concluded that true blood sugars are a better guide in interpreting glucose tolerance. 200 determinations (mostly on diabetics) of arterio-venous blood sugar difference revealed a variation from minus 26 to 102 mg. per 100 cc. Although it has been stated that venous blood sugar is higher than arterial blood sugar in diabetics, this was observed in only nine per cent of the tests. The average arterio-venous blood sugar difference in fasting normals and diabetics was 9 mg. per 100 cc. and this difference was not found to vary in diabetics with or without insulin two hours after a meal. However, the arterio-venous difference increased in both diabetics and non-diabetics after 100 Gms. of glucose was administered. The authors felt that the arterio-venous difference is a measure of sugar metabolized in the forearm, and therefore venous blood samples should be taken to indicate the over-all efficiency of carbohydrate metabolism. It was reasoned that arterial blood samples are preferable to determine renal glycosuria.—*H.S.G.*

PETERS, J. P. The use of carbohydrate in diabetic acidosis. *Am. J. Digest. Dis., 13* (5): 127–128 (1946).

The author advocated the intravenous administration of glucose (in addition to insulin) in cases of diabetic acidosis and coma. Reasoning that the high blood sugar (derived from tissue protein and fat) gives an exaggerated impression of the amount of glucose in the body and that the liver is probably deglycogenated in diabetic acidosis, he favored the use of parenteral glucose 1) to compensate for the glucose lost in the urine, 2) to accelerate the synthesis of liver glycogen which he felt is necessary before the patient can burn carbohydrate, and 3) to prevent possible hypoglycemic shock resulting from insulin administration. He treated patients in diabetic acidosis with 25 Gms. of glucose (10 per cent solution) intravenously and 50 units of insulin as soon as they were seen. Then glucose was administered (10 Gms. per hour) until the blood sugar

began to fall. With this, the rate of glucose administration was increased. Large amounts of saline were given subcutaneously.—*H.S.G.*

PARATHYROID

GILL, D. Multiple myeloma simulating hyperparathyroidism. *Ann. Int. Med. 24:* 1087–93 (1946).

A case of multiple myeloma with marked hypercalcemia and normal parathyroid glands is reported.—*J.M.*

THYROID

DOBYNS, B. M., Studies on exophthalmos produced by thyrotropic hormone. II. Changes induced in various tissues and organs (including the orbit) by thyrotropic hormone and their relationship to exophthalmos. *Surg., Gyn. & Obst. 82:* 609–17 (1946).

The administration of thyrotropic hormone to guinea pigs results not only in exophthalmos but in anatomic changes in a variety of tissues and organs. There is a generalized alteration of fat depots, which are rapidly depleted of their fat contents and are replaced by a more translucent, gelatinous material. Histologically these changes are characterized by the presence of edema and the infiltration of large numbers of polymorphonuclear leukocytes, lymphocytes and tissue macrophages. Early in the reaction the polymorphonuclear leukocytes and tissue macrophages are loaded with tiny fat droplets. Later on increase of new connective tissue is evident. Skeletal and cardiac muscle fibers during the early period of administration of thyrotropic hormone lose their striations and are found to contain multitudes of tiny fat droplets, which line up in a pattern representing the cross-striations of the fibers. Large amounts of fat are also found in the liver, kidneys and epithelium from several locations, as well as large phagocytic cells of the lungs, spleen and lymph nodes. These changes take place regardless of the presence or absence of the thyroid.—*J.M.*

DOBYNS, B. M. Studies on exophthalmos produced by thyrotropic hormone. III. Further study of changes induced in fat by thyrotropic hormone (tissue reactions associated with exophthalmos). *Surg. Gyn. & Obst. 82:* 717–22 (1946).

The sequence of events in the histologic changes induced in the connective tissue and liver of guinea pigs to whom thyrotropic hormone has been administered is described in detail. The earliest cellular reaction is composed principally of polymorphonuclear leukocytes and macrophages but later lymphocytes and fibroblasts which had transformed from macrophages are most numerous. These features develop simultaneously with exophthalmos. The sequence of appearance of fat in liver cells was demonstrated first as tiny fat droplets adjacent to blood sinusoids and later occupying the entire cell. The plasma fat and blood acetones become elevated simultaneously with the changes in the lipoid content of many organs. Associated with these changes is a polymorphonuclear leukocytosis. These cells were found to contain an excessive number of fat droplets in contrast to cells in the blood stream of normal animals.—*J.M.*